# ASSESSING MYCORRHIZAL INOCULUM POTENTIAL OF SOIL AND RESPONSE OF COWPEA TO INOCULATION ON A TROPEPTIC EUTRUSTOX

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By

John N. Awemo

Thesis Committee:

James A. Silva, Chairman
Goro Uehara
Charles L. Murdoch

We certify that we have read this thesis and that, in our opinion, it is satisfactory in scope and quality as a thesis for the degree of Master of Science in Agronomy and Soil Science.

THESIS COMMITTEE

James A. Silva
Chairman

Geo Uehara

Charles L. Murdoch

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

# I. INTRODUCTION

The rhizosphere of plant roots is usually colonised by a large number of soil-inhabiting micro-organisms including fungi which interact with the plant. In some cases the plant root is infected by parasitic fungi which cause the host tissue to become disorganised resulting in disease and eventual death of the plant. However, not all contacts between fungi and plants results in disease or death of the latter. In the course of time, a partnership between fungi and most plants has developed in which the roots become infected and an intimate balanced relationship beneficial to both is established. These root-fungus associations are called mycorrhiza and few plants growing under natural conditions are non-mycorrhizal. A mycorrhiza is initiated when the roots of plants become infected by a soil-borne symbiotic fungus. The fungus subsequently develops upon and within the root leading to the formation of an organ that is part fungus, part root.

Most plants form symbiotic associations with these fungi. Typically, the fungal spores germinate, infect the roots of the host plant and form characteristic structures called vesicles and arbuscules inside the root. Outside the root, an extensive network of hyphae spreads profusely into the soil. Mycorrhizal associations have been known

and well documented for about 100 years. However, inspite of their abundance and near-omnipresence in most soils and plant species, VA mycorrhizae have received little attention. It is only relatively recently that agronomists and soil scientists have started to focus their attention on mycorrhiza. A major factor that has contributed to the lack of interest and inhibited studies of the practical aspects of mycorrhiza is the inability to culture the fungus on artificial media in the absence of living plant roots. A factor responsible for the present heightened interest in VA mycorrhiza is the fact that recent research has indicated that mycorrhiza play a central role in the mineral nutrition of most plants of economic and agricultural importance; this is particularly true where soil phosphorus levels are low as is often the case in tropical soils. Improved plant nutrition due to mycorrhiza is not limited to phosphorus alone, since absorption of Zn, Cu, S, and K may also be enhanced.

In the tropics, the human population continues to increase at a rate which increases the pressure on the land and an already less-than-adequate food supply. New emphasis is being directed toward increasing and sustaining food production on tropical soils. One way this is done is with the use of suitable fertilizers in conjunction with suitable management practices. Inputs for increasing productivity are absent or minimal for farmers in many

developing countries. The greatly increased cost of phosphate as well as other fertilizers places great limitations on small farmers who need these fertilizers to increase crop yields. This has necessitated a search for possible ways to reduce dependence on the use of phosphate fertilizers or to increase the efficiency of their use by utilizing new and less expensive sources such as rock phosphate which abounds in many parts of the tropics.

It is known that when phosphate is added to soil it is subject to rapid fixation and therefore is not readily available to plants. Consequently, soils that are regularly fertilized with phosphate fertilizers develop large reserves of residual phosphate. How efficiently soil residual P is used by plants is not known. Therefore, any strategy that will give plants access to tightly bound P in soil will be very valuable. Vesicular-arbuscular mycorrhiza offer the prospect of increased use of slowly soluble forms of phosphate.

Additionally, mycorrhiza are an important and responsive part of the soil environment and agronomists and soil scientists are challenged to understand their ecology and interaction to the extent that agricultural practices may be adopted or developed to take full advantage of their potential. The objectives of this study are:

1. To investigate the relationship of soil mycorrhizae inoculum level to infection.

2. To evaluate the contribution of vesicular-arbuscular mycorrhiza to the growth of cowpea on a Tropeptic Eutrustox.

# II. REVIEW OF LITERATURE

## Terminology

The term mycorrhiza was first used by Frank (1885) to describe the occurrence and morphological formation of a fungus on the roots of trees. Since then the term has been used in various ways and with broader connotations. For example nodules on the roots of some plants caused by Rhizobia have been called mycorrhiza (Hiltner, quoted by Kelly, 1931) and fungus unions with tissue not of roots such as mosses and liverworts have also been called mycorrhiza. (Beauverie, 1902). Kelly (1931) after noting the variant uses of the term insisted that 'mycorrhiza' be used in the strict morphological sense as originally specified by Frank.

On the basis of their morphological features, three kinds of mycorrhizas can be distinguished (Peyronel et al., 1969). These are a) Ecto-mycorrhiza in which the root is entirely surrounded by a well-developed, compact mantle of fungal mycelia from the interior of which hyphae arise and pass into the root, b) Endo-mycorrhiza wherein the fungus grows within the root cells and the mycelia external to the root are poorly developed and do not form a mantle, c) Ectendo-mycorrhiza which are intermediate between the Ecto and Endo-mycorrhiza and possesses some of

the features of both of them.

Based on the nutritional interactions in the various kinds of mycorrhizae, Lewis (1973) proposed that the terms Ecto and Endomycorrhiza be abolished in favour of descriptive terms such as "Sheathing," "vesicular-arbuscular," "orchidaceous," and "ericaceous." The endomycorrhizae are divided into 2 groups: a) those produced by septate fungi and b) those produced by non-septate fungi. The latter are called Phycomycetous or Vesicular-arbuscular (VA) mycorrhiza and belong to the family Endogonaceae (Dowding, 1959; Mosse, 1956).

## Classification

Based on the work of many researchers (Tinker, 1975; Mosse, 1973a) it has become accepted to place the VA mycorrhizal fungi in the genus Endogone. Butler (1939) reviewed all the available evidence on the occurrence and systematic position of vesicular-arbuscular mycorrhiza and pointed out the close relationship of mycorrhizal fungi to the family Endogonaceae. He observed a resemblance (size and content) of the vesicles of these fungi to the chlamydospores of Endogonaceae and concluded that they probably belonged to the Endogone genus which have lost their power of forming fruiting bodies (sporocarps) common to the family. The genus Endogone is not well known. The history of the taxonomy of the genus was reviewed by Gerdemann and Trappe (1975). It had previously been

described by Thaxter (1922). According to this classification all species form sporocarps and are distinguished on the basis of spore character, structure of the sporocarp and general resemblance in habit. These spores may be formed in the soil either singly or aggregated in groups (Gerdemann and Nicolson, 1962).

In 1953 Mosse found sporocarps of a hitherto undescribed Endogone species attached to mycorrhizal strawberry roots and demonstrated a connection between these fruiting bodies and the endophyte in the roots. Using the sporocarps and spores excised from them, typical mycorrhizal infections were produced on strawberry, apples, and other plants (Mosse, 1956). This species was later named Endogone mosseae by Nicolson and Gerdemann (1968) in her honor. Gerdemann (1955) extracted from field soil large spores of an Endogone species which was later named Endogone gigantea by Nicolson and Gerdemann in 1968. In 1974 Gerdemann and Trappe surveyed the Pacific Northwest, USA and revised the taxonomy of the genus Endogone to include the genera Endogone, Glomus, Gigaspora Acaulospora, Sclerocystis and Glaziella. They provided keys for the identification of these genera. Since then several other genera have been described by Nicolson and Schenck (1979), Becker and Gerdemann (1977), Redhead (1977), Trappe (1977) and Gerdemann and Bakshi (1976). Hall and Fish (1979) proposed a key to the Endogonaceae

which was compiled using a computer program. The program assigned reliability weights to diagnostic characteristics on a scale of 1 to 9. High weights were assigned to characters which varied least and were easily observed.

## Occurrence and Distribution

The most widespread mycorrhizas are the vesicular-arbuscular type. They are not restricted to any group of plants. They are the rule rather than the exception in perennial plants under natural conditions, are less common in annual flowering plants and occur also in many ferns and liverworts (Mosse, 1963). This fact led Wilhelm (1966) to state that ". . . under agricultural field conditions, crops do not, strictly speaking, have roots, they have mycorrhizae." The point was underscored by Gerdemann (1968) when he stated that ". . . it is easier to list the plant families in which it is not known to occur than to compile a list of families in which it has been found." Vesicular-arbuscular mycorrhiza is world wide in their distribution, both under natural conditions and in cultivation. Geographically, it occurs on plants from the Artic (Katenin, 1963) to the tropics (Johnson, 1949). It can be found in most habitats. Gerdemann (1968) and Harley (1969) reported that aquatics and plants growing in wet places are most likely to be non-mycorrhizal. However, some workers have reported the occurrence of VA mycorrhiza in aquatic plants (Mason, 1928; Bagyaraj et al., 1979b; Sondergaard

and Laegaard, 1977). The leguminosae (Jones, 1924) and Graminae (Nicolson, 1959) are two families of great agricultural importance in which VA mycorrhizae have been reported to occur generally. Plant species reported to have mycorrhiza include citrus, tea, coffee, rubber, groundnuts, pigeon pea (Butler, 1939), oil palm, coconut, cocoa, banana, tobacco, tomato (Laycock, 1945), sugar cane (Ciferri, 1928), cotton (Sabet, 1939), maize (Gerdemann, 1968), apples (Mosse, 1957), avocado (Ginsburg, 1965), onion (Sanders and Tinker, 1971), yam (Johnson, 1949), pineapple (Mosse, 1981), soybean (Ross and Harper, 1970), barley (Owusu-Bennoah and Mosse, 1979), cassava (Yost and Fox, 1979), rice (Sanni, 1976b), potato (Black and Tinker, 1977), wheat (Khan, 1975), cowpea (Sanni, 1976a), papaya (Ramirez et al., 1975). Some plant species have been reported to be non-mycorrhizal. Members of the Cruciferae family such as cabbage generally do not form mycorrhizal associations (Gerdemann, 1968). Also included are members of the Chenopodiaceae and Cyperaceae (Mosse and Hayman, 1980).

From the wide range of crops having mycorrhiza, it is obvious that if mycorrhizal infections have even a slight direct or indirect effect on plant growth, their economic importance can be considerable.

There is very little host specificity among VA mycorrhiza and this probably accounts for its widespread distribution in plant species. However, there may be a difference in

the ease with which different host species become infected, the extent to which this occurs and the degree to which any one host is infected by different mycorrhizal fungi (Hayman, 1975; Strzemska, 1974). Apart from this difference in susceptibility, there is a quite remarkable lack of host specificity among different VA mycorrhiza. Thus Mosse (1973a) lists 20 host species which can be infected by the mycorrhizal fungi called "yellow vacuolate" (Glomus mossae). Gerdemann (1955) used large spores collected from the rhizosphere of red clover to inoculate various crops and found that a wide range of the crops inoculated were infected with the fungus. Mosse (1962) successfully established infections of VA mycorrhiza using an inoculum of germinating resting spores of an Endogone on a wide range of crops viz Trifolium parviflorum, T. glomeratum, T. pretense, T. subterraneum, Dactylis glomerata, wheat (Triticum vulgare) and onion (Alium cepa), a similar wide range of infections was reported by Ham (1962) using a species of Pythium isolated from mycorrhizal roots of Alium ursinum and lettuce.

## Assessment of Soil Infectivity

While the occurrence of mycorrhizal fungi has been reported in many soils, there is little information on either infection potential or spore numbers in the soil. There is therefore interest in surveying soils to identify the population of mycorrhizal fungi as well as to assess

infectivity efficacy. Information from several countries encompassing a wide range of natural and agricultural ecosystems has provided insight regarding the ecological significance of VA mycorrhiza. An important consideration in ecological studies is the accurate determination of the inoculum level in a soil. Present techniques used to assess inoculum levels include 1) extracting and counting spores; 2) direct observation of infection levels in plant populations; 3) measuring the rate at which a test seedling becomes infected; and 4) determining the maximum dilution at which infection occurs (Mosse, 1981). The first two methods are commonly used and, as Mosse points out, the method chosen depends on the purpose of the survey.

Mycorrhizal spores are usually extracted from the soil by wet-sieving and decanting (Gerdemann and Nicolson, 1963) or by a flotation-adhesion method (Sutton and Barron, 1972) and then the spores are counted under a microscope. Centrifuging spore extracts in a sucrose solution (Ohms, 1957) and differential sedimentation on a gelatin column (Mosse and Jones, 1968) have been used to improve separation of spores from organic debris and have proved to be an effective measure of spore population. Spore numbers in the range of 0.1 to 5 spores per gram of soil have been reported (Gerdemann, 1968; Nicolson, 1967; Schenck and Hinson, 1971; Mosse, 1981). Hayman (1970) recovered spore populations ranging from 2-120 per 50 gram sample in soil

from a Rothamsted wheat field. Sutton and Barron (1972), using the flotation-adhesion method recovered spore populations from the soil which were 10 - 100 times greater than those recovered by other methods. However, in this method it is believed that more dead spores are collected (Mosse, 1981).

Based on differences in spore numbers found in different soils, some workers have investigated whether or not the distribution of VA mycorrhiza is different in cultivated and non cultivated soils. In one such study Mosse and Bowen (1968b) reported higher numbers of spores in cultivated than under natural vegetation in 250 Australian and New Zealand soils. It was inferred from this that spores increase in cultivated soil as a result of intermittent root growth (Moorman and Reeves, 1979). Spore populations in the soil have also been reported to vary considerably for the same crop at different locations e.g., from 74 - 464 spores/100 g soil for wheat (Hayman and Stovold, 1979). The implications regarding soil infectivity are of great importance in view of these reports on variability of mycorrhizal populations in different soils, crops, varying environmental conditions, season, etc.

The relationship of soil inoculum to infection needs to be further investgated because although VA endophytes are present in most natural soils, there is evidence that sub-optimal levels in some soils lead to low infectivity.

This necessitates application of large quantities of P fertilizers during the early stages of growth for many crops such as cotton (Mosse, 1981). The low inoculum levels of some soils have been ascribed to low sporulation of the fungi. Mosse (1977a) observed that the inoculum density in the soil rather than its phosphate status determines responses to mycorrhizal inoculations. The inoculum level of 3 soils in Nigeria was found to be so low that Stylosanthes guyanensis and maize (Zea mays) did not become infected in the course of an experiment (Mosse, 1977a). Moorman and Reeves (1979) showed that the mycorrhizal population in the soil is greatly reduced when the top soil is severely disturbed. This finding is of vital importance in the tropics where the top soil is subject to severe erosion. Results from many studies have confirmed that VA mycorrhiza are present only in small numbers in eroded soils due to loss of top soil in which biological activity occurs (Powell, 1980). Reeves et al. (1979) reported that more than 99% of the plant cover in an undisturbed soil was mycorrhizal whereas less than 1% of the plant cover in the adjacent disturbed area (roadbed) was mycorrhizal.

The implication of these findings is that loss of top soil through erosion leaves the soil depleted of VA mycorrhizal fungi, and the inoculum level of the exposed soil may be sub-optimal for plant infection. Under these circumstances, it will be worthwhile to inoculate plants

with efficient mycorrhizal fungi. This practice will also be necessary in reclamation and revegetation programs (Reeves et al., 1979).

Mycorrhizal development and infectivity are affected by a number of factors including nutrient availability, season, soil moisture, light intensity and landscape (Tinker, 1975). These factors may act directly on the fungus or indirectly through their effect on the host. Interaction between fungi, the host species and conditions in the soil environment do not permit clear-cut generalizations. The system is complex. Most measurements of soil infectivity are usually made by estimating the proportion of potential host tissue (primary cortex) colonised by the fungus. This requires the collection of representative root samples which are stained by the method of Phillips and Hayman (1970). Hayman (1970) estimated both the incidence and extent of infection in the root system by measuring the length of infected root in each segment, percent root pieces with mycorrhizal infection and the percentage of root pieces with attached Endogone hyphae, spores or vesicles. Porter (1979) used the most probable number (MPN) technique (or method of ultimate dilution) for enumerating infective mycorrhizal propagules in the soil. In this method serial dilutions of non-sterile and sterile soil were used. Inoculum level was shown by the presence or absence of any VA mycorrhiza among roots that had grown through an aliquot.

Accuracy of this technique depends on the assumptions that 1) infective propagules are randomly distributed throughout the soil, 2) every infective propagule in the inoculum will produce a typical mycorrhiza during the test period and 3) there is no contamination.

## Effects on Plant Growth

The fact that most natural soils contain VA endophytes raises the question of whether or not they may be utilized to increase crop yield by pre-inoculating plants with them. Under field conditions the advantage conferred by pre-inoculation may be minimal due to the presence of indigenous endophytes in the soil; however, this depends on the fertility status of the soil. Since Frank's (1885) recognition of the symbiotic relationship between plant roots and mycorrhizal fungi, there has been much speculation about the significance of this relationship. Mycorrhiza were originally viewed by Frank as beneficial to the plant, aiding it in the absorption of water and nutrients. This concept was challenged by other workers, among them Hartig (quoted by Zag, 1964) who considered the mycorrhizal state to be purely a condition of parasitism on the part of the fungus. In his review of the significance of endotrophic mycorrhiza, Harley (1950) wrote: "It is impossible to accept the argument that all infections which have been called mycorrhizal infections benefit their host." He cited some cases which suggest that vesicular-arbuscular mycorrhiza had a deleterious

effect upon its host. Jones (1924) who worked with legumes also gained the impression that mycorrhizal infections were injurious to plants. Since then much has been done to establish the importance of mycorrhiza in plant growth.

The prevailing view today is that mycorrhizae are beneficial to plant growth. Mosse (1957) reported experiments in which mycorrhizal infections greatly increased growth of apple seedlings compared with plants under the same conditions, but without infection. Khan (1972) transplanted mycorrhizal and non-mycorrhizal maize seedlings to an unfertilized natural soil low in spore numbers (60/100 g soil) and previously occupied by weeds of the Chenopodiaceae family reported to be non-mycorrhizal (Gerdemann, 1968). Mycorrhizal plants grew better,took up more P and had grain yield that was 12 times greater than the control plants.

## Enhancement of P Uptake

The beneficial effect of mycorrhiza on plants growing in soils of low nutrient availability, especially low P, has been demonstrated by many workers and this favorable response has been attributed to improved phosphate uptake (Gerdemann, 1964; Baylis, 1959; Tinker, 1975). This has been shown to occur with onions and coprosma (Hayman and Mosse, 1971), maize (Gerdemann, 1964), strawberries (Holevas, 1966), soybeans (Ross and Harper, 1970), wheat (Khan, 1973) citrus (Kleinschmidt and Gerdemann, 1972), and tomato (Daft and Nicolson, 1966). Phosphate is relatively immobile

in soil and is present in the soil solution in very low concentrations. It is supplied to the plant mainly through diffusion. Roots have the potential of taking up P faster than it can be supplied by diffusion so that the concentration of P in solution near the root surface is greatly reduced. This results in a P depletion zone near the root surface (Bieleski, 1973).

Hayman and Mosse (1972) offered 2 possible mechanisms by which mycorrhizal plants obtain the extra P compared to non-mycorrhizal plants: 1) hyphae of mycorrhizal roots may absorb $PO_4$ from insoluble forms in the soil which are not available to non-mycorrhizal plants and 2) hyphae of mycorrhizal plants extend into the soil and absorb soluble phosphate beyond the phosphate depletion zone near the root surface. Most evidence supports this latter role. Sanders and Tinker (1973) conducted experiments on phosphate flow into mycorrhizal onion roots and concluded that the increased efficiency of mycorrhizal plants in the absorption of P was due to uptake and transport of $PO_4$ by the hyphae in the soil. Hattingh et al. (1973) obtained evidence to confirm this theory. Segments of mycorrhizal onion roots had high levels of radioactivity when $^{32}P$ labelled phosphate was placed in the soil 27 mm from the root surface Non-mycorrhizal roots had little radioactivity. When the hyphae from mycorrhizal roots were severed, mycorrhizal roots did not differ significantly in $^{32}P$ labelled phosphate

content from non-mycorrhizal roots. They concluded that the hyphal network enabled the plants to obtain $PO_4$ from a larger volume of soil extending beyond the depletion zone. These results also indicated that both mycorrhizal and non-mycorrhizal plants utilize the same source of soil phosphorus. Data such as these lend support to the theory of mycorrhizal effect, namely, that the hyphae form a more widely distributed surface for P uptake from the soil than plant roots alone.

Ross and Gilliam (1973) supplied phosphate sources of different availability to mycorrhizal and non-mycorrhizal plants and concluded that the principal source of $PO_4$ utilized by mycorrhizal plants was the one most readily available in the soil. Hayman and Mosse (1972) showed that $NaHCO_3$-extractable (available) phosphorus in the soil was the chief source of phosphorus for both mycorrhizal and non-mycorrhizal plants. These results are inconsistent with the findings of Daft and Nicolson (1966) and Murdoch et al. (1967) who showed that VA mycorrhiza improved the growth of plants more when the phosphate source was less-readily available, e.g., rock phosphate or apatite. Mycorrhizal and non-mycorrhizal maize grew equally well when the phosphate source was readily available, e.g., superphosphate or monocalcium phosphate. When the phosphorus was slowly avaiable (rock phosphate, apatite) mycorrhizal maize grew better and took up more P than non-mycorrhizal

plants. It has been suggested by some workers that mycorrhizal fungi may be involved in the solubilization of the less soluble forms of P in soil enabling mycorrhizal plants to utilize forms of P unavailable to non-mycorrhizal plants (Hattingh et al., 1973). Stout and Overstreet (1954) showed that the phosphate in solution was renewed 10 times a day during the growth of plants in soils containing 1 ppm phosphate in solution. Therefore the exploration of a greater volume of soil by mycorrhizal plants should result in the release of more $PO_4$ into the soil solution. It is believed that this is the mechanism by which mycorrhizal plants are able to use less soluble forms of phosphate more efficiently (Gerdemann, 1975).

Phosphate supply to roots and its uptake by plants has been extensively studied and the depletion zone near the immediate vicinity of the roots has been demonstrated by many investigators (Bhat and Nye, 1974; Rhodes, 1979). Owusu-Bennoah and Wild (1979) used autoradiography to study phosphate depletion zones around mycorrhizal and non-mycorrhizal roots. They showed that most P uptake by mycorrhizal roots was from soil within 2 mm of the root surface. These results differ from those of both Hattingh et al. (1973) and Rhodes and Gerdemann (1975) who showed that mycorrhizal hyphae can extend up to 2.7 and 7 cm from the root surface, respectively. The amount of external hyphae and the distance to which they extend are important

factors determining $PO_4$ uptake from the soil by mycorrhiza. Sanders and Tinker (1973) estimated that there were 80 cm of mycorrhizal hyphae per cm of infected onion roots. Hattingh (1975) suggested that the condition of the soil in this latter experiment may have induced more profuse hyphal growth than is possible under normal field conditions. Cooper and Tinker (1978) studied the uptake and translocation of P, Zn, and S in mycorrhizal clover (Trifolium repens) and onion (Allium cepa). They showed that for clover, external hyphae translocated molar amounts of P, Zn and S in the ratio of 35:5:1 and the mean flux in the ratio of 50:8:1 which suggests higher relative efficiency in the uptake and translocation of P. Their results also suggested that translocation in the hyphae is an effective process under metabolic control and that the amount of nutrient transported in the hyphae may be controlled by the demand of the plant rather than the amount of external hyphae in the soil.

Baylis (1972) has suggested that mycorrhiza have exercised a controlling influence on the evolution of roots and that plant species deficient in root hairs are more dependent on mycorrhiza or added phosphate for growth in P deficient soils than plants with extensive root systems and copious root hairs.

## Effects on Nodulation by Rhizobium

Research by many workers has shown that VA mycorrhizae

play an important role in nodulation and symbiotic nitrogen fixation. In many experiments in P deficient soils, nodulation and nitrogen fixation failed to occur unless the plants were either inoculated with VA mycorrhiza or supplied with liberal amounts of phosphate (Crush, 1974; Daft and El Giahmi, 1974, 1976; Mosse, Powel and Hayman, 1976). Enhanced nodulation and $N_2$-fixation by mycorrhiza in legumes is attributed to improved P supply. Bergerson (1971) and Munns (1977) showed that symbiotic $N_2$-fixation has a high phosphate requirement.

## Effects on Water Uptake

VA mycorrhiza can also enhance the plant water uptake from the soil. Safir et al. (1971, 1972) measured the rate of water uptake in soybeans subjected to water stress and found that mycorrhizal plants recovered more rapidly than non-mycorrhizal plants. There was no difference in water movement in stems and leaves of mycorrhizal and non-mycorrhizal plants indicating that the main effect of mycorrhiza was to enhance water transport in the roots. Application of nutrients to the soil essentially eliminated differences in water uptake between mycorrhizal and non-mycorrhizal plants indicating that the decreased resistance to water transport in mycorrhizal plants was due to an increase in nutritional status of the host brought about by mycorrhiza. It has also been suggested that decreased resistance to water transport in mycorrhizal plants may

be caused by changes in the morphology of the plant (Gerdemann, 1975). The finding of Daft and Okusanya (1973) that mycorrhizal infections increase the amount of vascular tissue in maize, tomato and petunia lend support to this view.

## Role in Disease Resistance

In addition to improved plant nutrition, there is indication that mycorrhiza may play a role in resistance to root diseases of plants. Of interest is the possibility that mycorrhizal fungi can protect roots from attack of parasitic fungi by a) utilizing surplus carbohydrates thus reducing attractiveness of roots to pathogens, b) producing a physical barrier and c) secreting antibiotics (Zag, 1964). Damage caused by Fusarium and other wilt diseases was reduced when plants were mycorrhizal (Dehne and Schonbeck, 1975). Also in the ectomycorrhizas, the root is completely covered by a thick mantle of tightly fitting hyphae and the cortical cells are surrounded by fungal mycelia. This can protect the root from pathogenic infection since to enter root tissue an invading pathogen must physically break and penetrate this barrier. In common with other soil fungi, mycorrhiza can produce antibiotics. Wilkins and Harris (1944) found that extracts from many basiomycetes including mycorrhizal species inhibited growth of bacteria. It has thus been suggested (Zag, 1964) that antibiotic production by mycorrhizal fungi may be a factor in the survival of

seedlings in nature by warding off attack by root pathogens.

# III. MATERIALS AND METHODS

## Experiment 1: Assessment of Soil Inoculum Potential

The Lahaina Taxadjunct (Clayey, Kaolinitic, isohyperthermic family of the Tropeptic Eutrustox) was used for this experiment. Soil material was collected from block K of the Molokai site of the Benchmark Soils Project from plots that had been fertilized with 2.5, 16.5 and 30.5 kg P/ha in June 1982. This site had previously been under pineapple cultivation and later under corn. Surface soil (0-15 cm) was collected from 4 randomly selected positions within each plot and mixed thoroughly by spreading it on a bench and turning it over several times. All soil material was passed through a 6 mm sieve. Soil pH was adjusted to 5.5 using $Ca(OH)_2$. Basal nutrients (KCl, 30 mg K/pot; $ZnSO_4 \cdot 7H_2$, 5 mg Zn/pot; $H_3BO_3$, 0.5 mg B/pot; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.5 mg MO/pot) were applied in solution to the surface of the soil and mixed thoroughly by turning the soil over several times. No Rhizobium inoculation was done. Each pot contained 500 g of soil. The soil were characterized by the following measurements: pH (1:1 soil to distilled water and KCl), available (Mod Truog) P, CEC, and exchangeable bases Ca, Mg, Na and K. Results of these measurements are presented in Table 1.

Table 1 : Chemical properties of soil (with residual P) used for assessment of mycorrhizal inoculum potential.

| Soil* | Mod.Truog P (ppm) | pH | | me/100g | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $H_2O$ | KCl | CEC | Ca | Mg | Na | K |
| 1 | 8.20 | 4.8 | 4.4 | 20.10 | 2.54 | 1.36 | 0.17 | 1.35 |
| 2 | 18.65 | 4.9 | 4.4 | 20.27 | 2.91 | 1.25 | 0.15 | 1.16 |
| 3 | 23.36 | 4.9 | 4.5 | 20.30 | 2.36 | 1.26 | 0.16 | 0.92 |

* 1, 2, 3 = Soils fertilized with 2.5, 16.5 and 30.5 KgP/ha respectively in June 1982.

Spore Extraction

The method of wet-sieving and decanting (Gerdemann and Nicolson, 1963) was used to extract spores from the soil. Fifty grams of soil were stirred in about 1 litre of water in a small plastic bucket and the heavier particles allowed to settle for a few seconds. Stirring was done by force of running tap water throug a small plastic tube attached to the top. The mixture was then decanted through a 63 micrometre sieve and the process repeated three times. The mixture of spores and debris on the sieve was suspended in 20 ml of a 30% sucrose solution in a centrifuge tube and centrifuged at 200 revolutions per minute for about one minute. The suspended spores were then transferred to an open petri dish and the spores picked out with a syringe and counted with the aid of a dissecting microscpoe.

Soil Dilution Bioassay

A portion of each soil was sterilized by gamma irradiation (2.5 Mrad) from a $^{60}Co$ source (Dept of Food Science and Human Nutrition, University of Hawaii) and used as the diluent in making 5 dilutions of non-sterile and sterile soil. The proportions of non-sterile to sterile soil were 1/0, 1/4, 1/16, 1/64, and 1/256. The quantities of non sterile and sterile soil used for each dilution are shown below.

| Dilution | Soil (grams) | |
|---|---|---|
| | Non-sterile | Sterile |
| 1/0 | 500 | 0 |
| 1/4 | 125 | 375 |
| 1/16 | 31.2 | 468.8 |
| 1/64 | 7.8 | 492.2 |
| 1/256 | 1.9 | 498.1 |

The dilution were made by weighing the required quantities of non-sterile and sterile soils and mixing them thoroughly in an inflated polyethylene bag. There were 4 replications per treatment.

After each treatment was mixed, it was divided equally into 4 pots to give 500 g of soil per pot. Pots were then arranged on benches in the greenhouse at the Mauka campus research facility of the Department of Agronomy and Soil Science in a randomised complete block design. Cowpea seeds (Vigna unguiculata L. var. 5227 Mississippi Purple) were surface sterilized by soaking them in clorox solution for about 5 minute and then washing them in distilled water. Three surface sterilized seeds were sown in each pot and thinned to 2 plants per pot 3 days after emergence. All pots were maintained at field capacity for the duration of the experiment by frequent weighing, with addition of water as needed. Plants were harvested 30 days after planting and the following measurements were made: plant height, fresh and dry weights of tops, fresh and dry weights of roots, percent infection of roots and chemical composition of leaves after drying to constant weight at

70°C for 24 hours and grinding. Nutrient analysis was done by X-ray Fluorescence Quantometer in the Dept. of Agronomy and Soil Science.

Assessment of Root Infection

Root infection was assessed by collecting representative root samples from the entire root system from 4 different portions of the root mass and combining them into one sample. Only the fine terminal feeder roots were sampled since these are the primary sites of mycorrhizal development (Kormanik and McGraw, 1982). The root samples were then cleared and stained according to the procedure described by Phillips and Hayman (1970) and examined in an open petri dish with a dissecting microscope (40-100 magnification) for the presence or absence of mycorrhizal infection.

Mycorrhizal infection was indicated by the presence of vesicles, arbuscules and hyphae on the root. The objective of the evaluation was to determine the approximate percentage of roots infected. This was done by spreading the cleared and stained roots uniformly in an open petri dish and carefully rotating the entire sample on the microscope stage to make a visual assessment of colonised roots on a scale of 0 - 100%.

## Experiment 2: Evaluation of Mycorrhizal Contribution to Growth of Cowpea

The Lahaina Taxadjunct (Clayey, Kaolinitic, isohyperthermic family of the Tropeptic Eutruxtox) was used. Soil

material was collected from two blocks in the Molokai site of the Benchmark Soils Project. One of these blocks had not been cultivated or fertilized for at least one year prior to the experiment. The other had been fertilized as described below. Surface soil (0 - 15 cm) was collected from 4 randomly selected positions within the chosen plots. The soil was thoroughly mixed by spreading it on a bench and turning it over several times. All soil was passed through a 6 mm screen. Soil pH was adjusted to 5.5 with $Ca(OH)_2$. Basal nutrients (KCl, 120 mg/pot; $ZnSO_4 \cdot 7H_2O$, 20 mg/pot; $H_3BO_3$, 2 mg/pot; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 2 mg/pot) were applied in solution to the surface of the soil and mixed thoroughly by turning the soil over several times.

Soil with Freshly Applied P

The soil material for this experiment was collected in block R which had not been cultivated or fertilized for at least one year and was occupied by various perennial weeds. The soil was characterized by the following measurements: pH (1:1 soil to distilled water and KCl), available (Mod Truog) P, CEC, KCl extractable Al, and exchangeable bases Ca, Mg, Na, and K. Results of these measurements are presented in Table 2. Five levels of P were established with the amount to be added determined from a P extraction curve (Figure 1). Monocalcium phosphate $Ca(H_2PO_4)_2 \cdot 2H_2O$ was used to supply P. Amounts added were

Table 2: Chemical properties of soils used for evaluation of the mycorrhizal contribution to cowpea growth.

| Soil* | Mod.Truog P (ppm) | pH | | me/100g | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $H_2O$ | KCl | CEC | Ca | Mg | Na | K | KCl Ext. Al |
| Unfertilized | 13.62 | 4.9 | 4.3 | 14.60 | 4.21 | 1.37 | 0.09 | 1.50 | 0.05 |
| Residual P* | | | | | | | | | |
| R1 | 14.80 | 4.8 | 4.2 | 15.26 | 3.54 | 1.30 | 0.09 | 0.94 | 0.14 |
| R2 | 15.32 | 4.7 | 4.1 | 14.81 | 3.50 | 12.9 | 0.10 | 0.90 | 0.15 |
| R3 | 20.58 | 4.8 | 4.3 | 15.15 | 4.00 | 1.45 | 0.11 | 0.04 | 0.06 |
| R4 | 21.95 | 4.6 | 4.1 | 15.05 | 3.21 | 1.31 | 0.08 | 1.17 | 0.24 |
| R5 | 25.86 | 4.8 | 4.2 | 15.34 | 3.46 | 1.42 | 0.09 | 1.11 | 0.10 |

* R1 - R5 = Plots fertilized with a total of 0, 25, 50, 65 and 105 KgP/ha respectively applied in two applications (January and June, 1981).

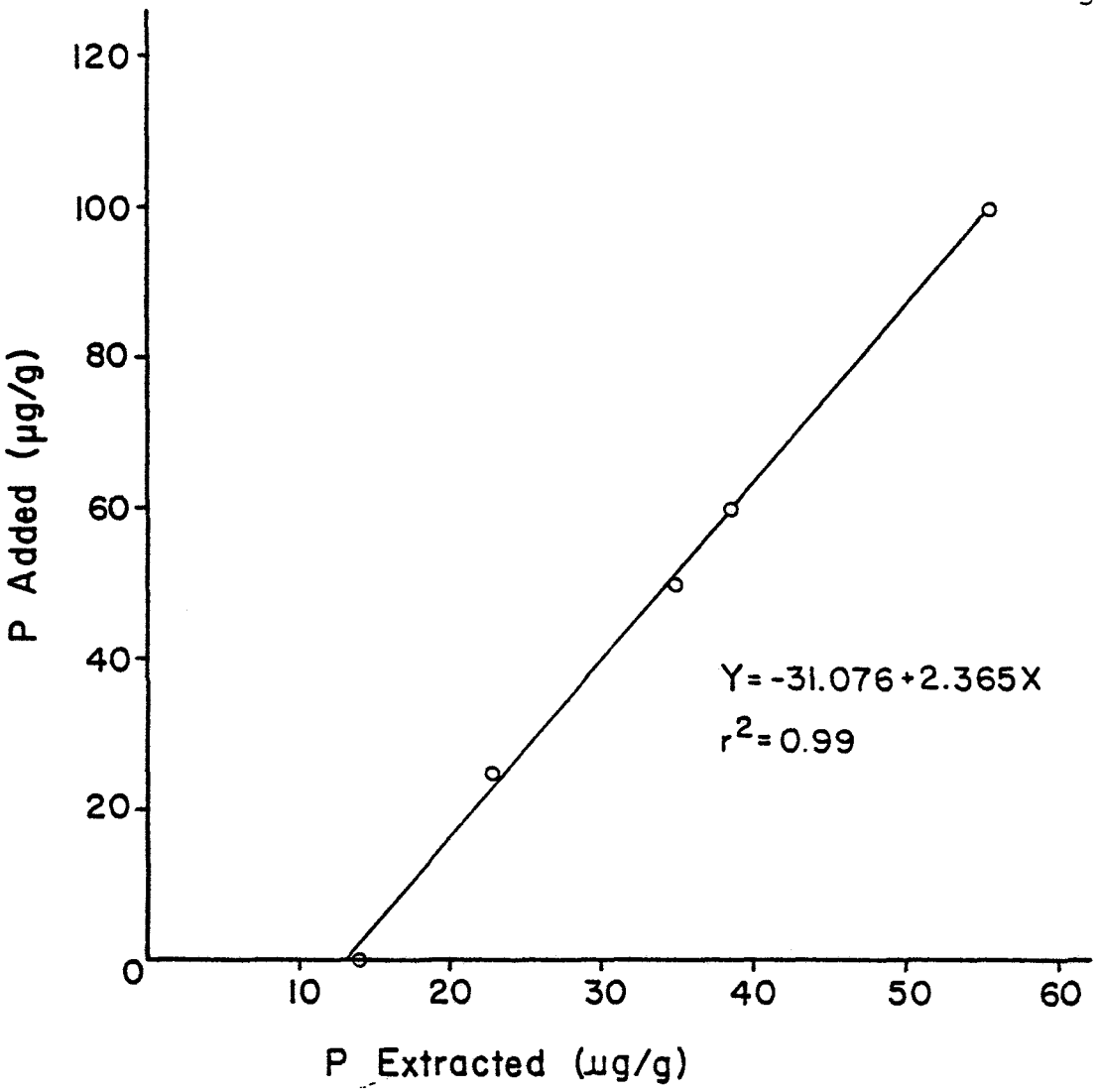


Figure 1: Modified Truog Phosphate extraction curve for the Tropeptic Eutrustox soil with newly applied P.

0, 12, 24, 48 and 96 µgP/g per pot of 2 Kg of soil. Half of the soil was fumigated using methyl bromide fumigant and half was left unfumigated. The unfumigated soil was included in order to determine whether or not indigenous mycorrhizal fungi are adequate to effect optimum infection and therefore allow maximum plant growth. Two Kg of soil were added to 4 l pots lined with plastic bags with holes at the bottom.

Three mycorrhizal (inoculated) cowpea treatments were established in half of the pots in each P treatment. Inoculation was done by placing 10 g of the inoculum beneath the seed at planting. An inoculum of Glomus mosseae in the form of infected corn roots which also included spores mixed with sterile sand was used. The remaining pots did not receive the inoculum. All treatments were also inoculated with a 3-strain Rhizobium mix for Vigna unguiculata supplied by NIFTAL. All plants were thinned to 2 plants per pot 3 days after emergence. Pots were arranged on benches in the greenhouse in a randomised complete block design. All pots were maintained at field capacity for the duration of the experiment by frequent weighing and addition of water as needed. All plants were fertilized with a nutrient solution containing Mg, S, Cu, Fe, Mn, Zn, B, and Mo once weekly to ensure uniform growth as described for experiment 1.

Plant height was measured at 30, 60 and 90 days. Plants

were grown to maturity and data collected on fresh weight of stover, fresh weight of roots, pod and seed yield, nodulation and chemical composition of leaves sampled at 50% flowering.

Assessment of Root Infection

Percentage of infection in root tissue was estimated on samples of fine root collected from each pot. To do this, soil was washed from the root system of plants in each pot and representative samples were taken from 4 different portions of the entire root system and combined. The samples were cleared with 10% KOH and stained with lactophenol + trypan blue after acidification with 10% HCl (Phillips and Hayman, 1970. Roots were spreaded in an open petri dish as described previously and examined microscopically. Infection was rated on a scale of 0 - 100% of the representative root samples.

Nodulation was rated visually on a scale of 0 - 10 with 0 = no nodules, 1 - 3 = few nodules, 4 - 6 = average number of nodules and 7 - 10 = abundant nodules.

Soil with Residual P

Soil for this experiment was collected from block K with a past history of pineapple cultivation and later sorghum. Plots which had 5 levels of residual P were sampled as described above. These plots had been fertilized with a total of 0, 25, 50, 65, and 105 Kg P/ha,

respectively, applied in 2 applications in January and June, 1981. Results of chemical analysis of this soil are presented in Table 2. The soil was sterilized and inoculation treatments given as described previously. Similar data were also collected.

Data Analysis

Analysis of variance was used to examine the data to identify significant effects and means were compared with Duncan's multiple range test. Regression analysis was used to determine the correlation among the variables.

# IV. RESULTS AND DISCUSSION

## Assessment of Soil Inoculum Potential

### Spore Count

The objective of this experiment was to investigate the relationship of soil inoculum level to infection. As explained in the materials and methods section,the procedures used for estimating soil inoculum potential were a) extraction and counting of spores, and b) serial dilution bioassay.

Results of the spore count are presented in Table 3. The soil was found to have an indigenous spore population of 5 spores per gram of soil. Spore numbers did not differ significantly in soils collected from the 3 plots with different P levels. On a soil volume basis the inoculum level of this soil may be regarded as fairly high and capable of causing reasonable infection of plant roots. Soils with spore numbers as low as 0.3 spores per gram of soil have been reported to produce infections ranging from 46-80% (Powell, 1980) while Daft and Nicolson (1969) found that 3 spores per plant were able to effect complete colonisation of roots.

Since mycorrhizal fungi are obligate symbionts (Gerdemann, 1968) they can survive only as resting spores in the soil in the absence of living host roots. The

Table 3: Mycorrhizal spore count in soil at three levels of residual phosphorus.

| Soil P Level µg/g | Spores $g^{-1}$ soil | Range |
|---|---|---|
| 8.20 | 5.2 | 4.9-5.4 |
| 18.65 | 4.7 | 3.9-5.4 |
| 23.36 | 5.1 | 4.3-5.8 |

relative abundance of spores in the soil has been used as a measure of VAM infectivity level (Hayman, 1970; Sutton and Barron, 1972). However, spores are not the only infective propagules in the soil. Hyphae from infected roots have been shown to cause infection (Powell, 1976) and Read et al. (1976) showed that infection arose from root to root contact rather than from spores. Redhead (1977) was unable to extract any spores from some Nigerian soils where many plant species were found to be strongly mycorrhizal. Another difficulty in relying on spore numbers as an index of soil inoculum level is that some VA endophytes produce spores too small to be extracted or counted and some may not produce spores (Porter, 1979). Moreover, even spores that are large enough to be extracted and counted may not all be recovered.

In addition, reports of the correlation between numbers of spores in soil and amount of root infection are not consistent. Both Hayman (1970) working with wheat and Daft and Nicolson (1972) working with maize found that the number of spores was related to root infection. On the other hand the number of spores present in soil was not found to be correlated with root infection by Mosse (1973a). Also data obtained from spore counts by Sutton and Barron (1972) and estimates of numbers of infected roots (Sparling and Tinker, 1975) show that the population of mycorrhizal fungi decrease with depth. Most spores

are found in the top 15 cm of soil. Often it is said that the subsoil is infertile. This infertility may not be due to lack of nutrients but to insufficient mycorrhiza.

Soil Dilution Bioassay

Assessment of infection in the roots constitutes an integral part in many studies involving VA mycorrhiza. Most assessments often involve estimating the approximate proportion of potential host roots occupied by the fungus.

Results of the soil dilution bioassay described in this experiment indicate that mycorrhizal infection progressively decreased as the amount of sterile soil in the dilution series increased (Figure 2). Incidence of infection was highest in the 1/0 and 1/4 dilutions and lowest in the 1/64 and 1/256 dilutions. Differences in infection among the various dilutions were significant at the 5% level (Appendix Table 25). Infection was also highest at the lowest P level and lowest at the highest P level. The fact that infection occurred at soil dilution as high as 1/256 suggests that the natural infectivity of this soil is quite high and reflects the fairly high spore numbers noted earlier. Also the similarity in percent infection between the two highest dilutions (1/64, 1/256) at the higher soil P levels may suggest that further dilution would probably result in very little or no decrease in infection. Porter (1979) found that there was good agreement between the number of endophyte propagules

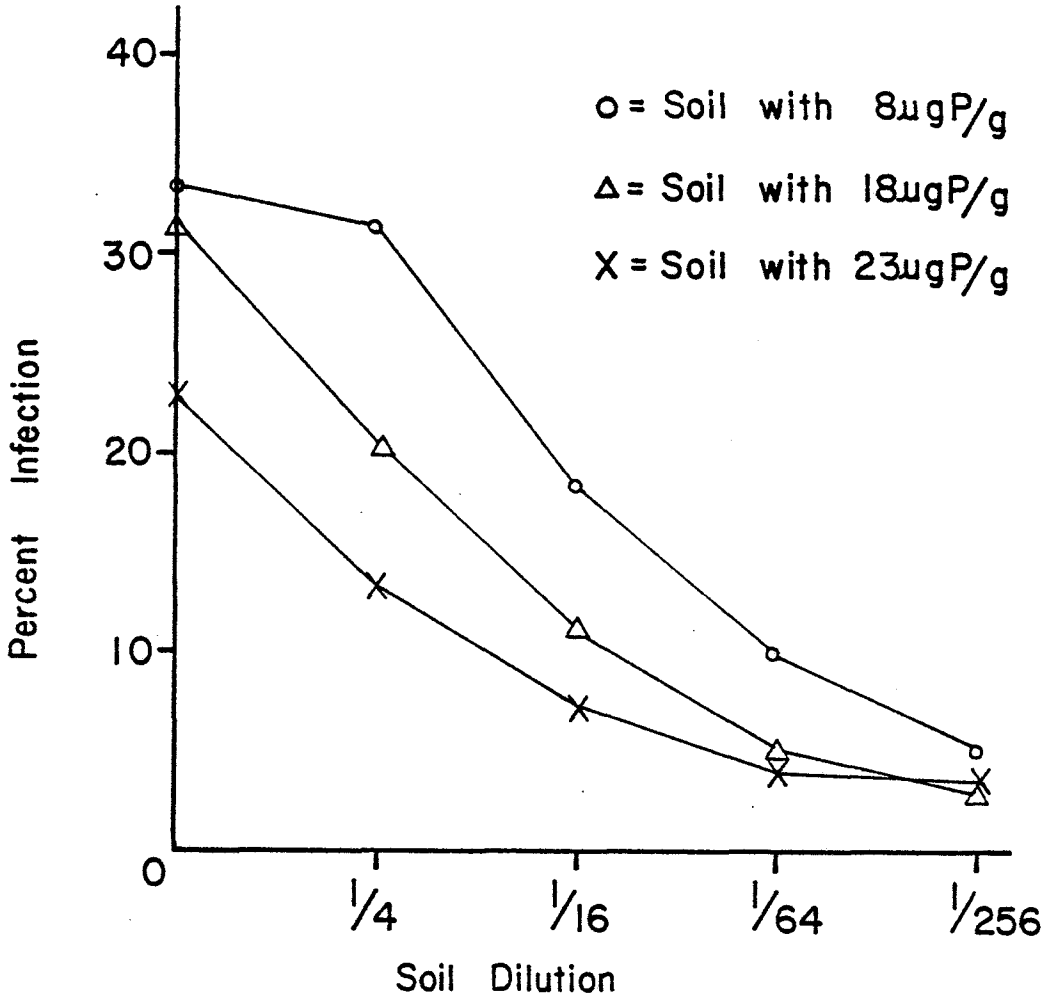


Figure 2: Incidence of mycorrhizal infection in roots of cowpea plants grown in a 5 dilution series of non-sterile and sterile soil at 3 P levels.

estimated by the soil dilution method and the number of spores extracted by the wet-sieving method.

Infection was observed to be erratic in general through out the range of dilutions used, but more so in the highest dilution (1/64, 1/256) than in the lowest dilution (1/0, 1/4). This was equally true whether the entire root system was considered or when a particular infected root segment was considered. External hyphae were absent or only poorly present in the 1/0 and 1/4 dilutions. No external hyphae were observed in the highest dilutions. Longitudinal internal hyphae and vesicles were present in all roots where infection took place. The test plants in this experiment were grown for 30 days and it seems that under the experimental conditions complete infection had not occurred. The sporadic incidence of infection observed probably corresponded to the 'infection units' which were described by Cox and Sanders (1974) as "a series of discrete units each relating to one entry point."

An interesting observation during this experiment was that plants growing in the highest dilutions (1/64, 1/256) withered within one week while those in the undiluted soil 1/0 and 1/4 dilution grew normally. This effect is attributed to residual atrazine action which had been used as a herbicide on the plot prior to the experiment. Atrazine is degraded by hydrolysis and the process is aided by micro-organisms. When the soil is sterilized or partially

sterilized as in this experiments the microorganisms are eliminated or reduced and hence residual action of atrazine persists for a longer time (Nishimoto, personal communication*). This condition was corrected with the incorporation of activated charcoal (1 g/pot of 500 g soil).The plants were uprooted and seeds were replanted after the treatment. It was also observed that plants growing in the undiluted soil (1/0) and 1/4 dilution exhibited N deficiency symptoms (yellowing of leaves, mostly lower leaves) while plants in the intermediate and high dilutions grew normally with well-formed green leaves. Growth was also observed to be less in the undiluted and 1/4 dilutions than in the intermediate and high dilutions. Reasons for these observations will be discussed later.

Baker (1965) and Wilhelm (1966) have discussed the theory of inoculum and inoculum potential of the soil. To the plant pathologist, at least two concepts are used in defining the term. In a broad sense it may refer to the "vigor of a pathogen to establish infection," or "the susceptibility of the host and amount of inoculum present." As it refers to VA mycorrhiza, inoculum potential means an inherent capacity to invade the host without causing disease (Wilhelm, 1966). The potentiality of VA mycorrhiza to colonise the host roots is a function of inoculum

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* R. K. Nishimoto--Department of Horticulture, Univ. of Hawaii.

density, that is, amount of inoculum per unit mass of soil.

Most measurements of infection are based on an assessment of the proportion of potential host roots occupied by the fungus. Usually measurements are made on representative root samples as was the case in the present investigation. However, measurement of inoculum potential of soil using host plants as indicators has some limitations. Such factors as susceptibility of the host to mycorrhizal infections, mass of roots present and environmental factors must be considered in addition to inoculum density. For example, a sudden change in the environment or vigor of the test plant may alter the amount of infection. It was evident during the course of this experiment that the test plants did not grow uniformly. Herbicide carry-over from the field caused depressed growth in some treatments. In addition, Nitrogen released from the soil with the highest dilution enhanced the growth of plants growing in these dilutions. Consequently the infection recorded might not reflect the actual infectivity potential of the soil because unequal amounts of roots were produced in the various dilutions. To obtain results that are comparable, conditions for growth of the test plants must be held as constant as possible.

Biermann and Lindermann (1981) have proposed a standard method for the quantification of mycorrhizal colonisation

of roots based on the percentage of the length of root segments containing VA mycorrhizal structures (vesicles, arbuscules or hyphae) rather than the determination of the percentage of infection of representative root samples. In their method the total root system was cut into 0.5 to 1.0 cm pieces and cleared and stained with trypan blue (Phillips and Hayman, 1970). A randomly selected aliquot of the stained root segments was spread in an open petri dish marked with 1 cm grids and examined under the microscope. The proportion of the length of each segment which contained mycorrhizal structures (vesicles, arbuscules or hyphae) was estimated as the frequency of percentage of root lengths with mycorrhizal structures to the total number of segments examined. Possible sources of error in estimating mycorrhizal infection include a) using non-representative root samples b) personal error of the observer since most assessments are subjective estimates, c) non-uniformity of growth conditions of the host and d) possible inhibition of mycorrhizal development in the rhizosphere, e.g., pH, nutrients, etc. Due to these limitations it is difficult to determine to what extent any method of assessment accurately measures the true level of infection.

The method of serial dilution was employed by Tsao (1960) for estimating the disease potential of citrus Phytophthora in the soil. The rationale of this method

was that serial dilution of unsterilized with sterile soil as the diluent would eventually reduce the disease potential to zero. This method works on the assumption that the infective propagules are randomly distributed throughout the soil. In practice this implies that the soil mixture is thoroughly mixed; therefore, this also means that the roots of a test plant growing in the soil are equally likely to be infected in any part of the soil. In this respect, sampling of roots for rating of infection is critical. The soil-root system is dynamic, with roots growing and spreading through the soil and becoming infected as they encounter viable propagules. The extent of infection depends not only on the number of viable propagules in the soil but also on the abundance of susceptible roots (Dowdle, 1980). The probability of a root encountering an infective propagule depends on the abundance of propagules per unit volume of soil. One difficulty in using the soil dilution method to estimate inoculum potential is that with high clay soils such as the one used for this experiment, strong aggregation and the presence of binding substances e.g., Fe and Al oxides may invalidate the assumption of random distribution of infective propagule. Spore extraction was rendered difficult because some of the spores, especially the small ones, were obscured by coatings of Fe oxide. Dispersion of the soil with sodium hexametaphosphate was often necessary.

Following the observation that plants in the undiluted soil and 1/4 dilution showed N-deficiency symptoms and also did not grow as large as plants in the higher dilutions, nutrient analysis of the leaf tissue was carried out. Data for percent nitrogen in leaves are presented in Table 4. Nitrogen content of the leaves increased as the proportion of non-sterile to sterile soil increased. Plant height also increased with increasing dilution of non-sterile with sterile soil (Table 5). It is worth noting that the opposite occurred in the case of root infection. The response, therefore, is not related to mycorrhiza, but can be attributed to increased release of N in the soil as the amount of sterilized soil in the dilution increased. The effect of gamma irradiation on the availability of N and P in soil has been investigated by Eno and Popenoe (1963, 1964) and by Singh and Kanehiro (1970). Both studies established the fact that gamma irradiation generally increased the release of N, P, and S in soil. This was shown by both soil and plant uptake measurements. The release of N and P in soil following gamma irradiation will have important effects on plant growth. Eno and Popenoe offer the following explanation for this increase: nitrogen and phosphorus are constituents of both living and dead plants and animals in the soil. The lethal effect of gamma irradiation causes the death of cells of microorganisms and the subsequent degradation of residues

Table 4: Nitrogen content (%) of cowpea leaves at 30 days in the dilution series of nonsterile and sterile soil at 3 levels of residual soil P. (means of 3 replicates).

| Dilution | Soil P Level (µg/g) | | |
|---|---|---|---|
| | 8 | 18 | 23 |
| | N(%) | | |
| 1/0 | 1.81 | 1.52 | 2.56 |
| 1/4 | 2.56 | 2.04 | 2.07 |
| 1/16 | 2.55 | 2.22 | 2.75 |
| 1/64 | 2.71 | 2.63 | 2.35 |
| 1/256 | 2.49 | 2.46 | 2.31 |

Table 5: Mean height (cm) of cowpea plants at 30 days grown in the dilution series of non-sterile and sterile soil at 3 levels of residual P (means of 3 replicates).

| Dilution | Soil P Level (µg/g) | | |
|---|---|---|---|
| | 8 | 18 | 23 |
| | cm | | |
| 1/0 | 12.9 | 16.8 | 11.1 |
| 1/4 | 13.3 | 16.5 | 16.6 |
| 1/16 | 15.9 | 18.1 | 13.2 |
| 1/64 | 14.4 | 14.1 | 15.2 |
| 1/256 | 14.1 | 14.5 | 15.8 |

results in increased amounts of N and P being released.

## Evaluation of Response of Cowpea to Mycorrhizal Inoculation

### Effects of Methyl Bromide Fumigation

#### Effects on growth

The effects of soil sterilization on soil biological and chemical changes have been studied by many workers. These studies have established relationships between these changes, availability of plant nutrients, plant growth and yield. Three commonly used sterilization processes are fumigation with various chemicals, autoclaving, and gamma irradiation. These processes have been shown to have both beneficial and harmful effects on plant growth.

In the present investigation fumigation with methyl bromide effectively destroyed the indigenous mycorrhiza from the soil as evident from the fact that all non-inoculated plants in the fumigated treatments remained non-mycorrhizal (Figure 3 and 4). In addition to eliminating mycorrhizal fungi, fumigation depressed the growth variables of all plants (Table 6) and this occurred regardless of the P level (Figure 5). The effect was particularly severe in the non-inoculated treatments where the plants made little or no growth. Only very small leaves were formed and they withered and dropped off after a few days. As a result, no leaf samples were available for tissue analysis except at the highest P levels. This also resulted in differences

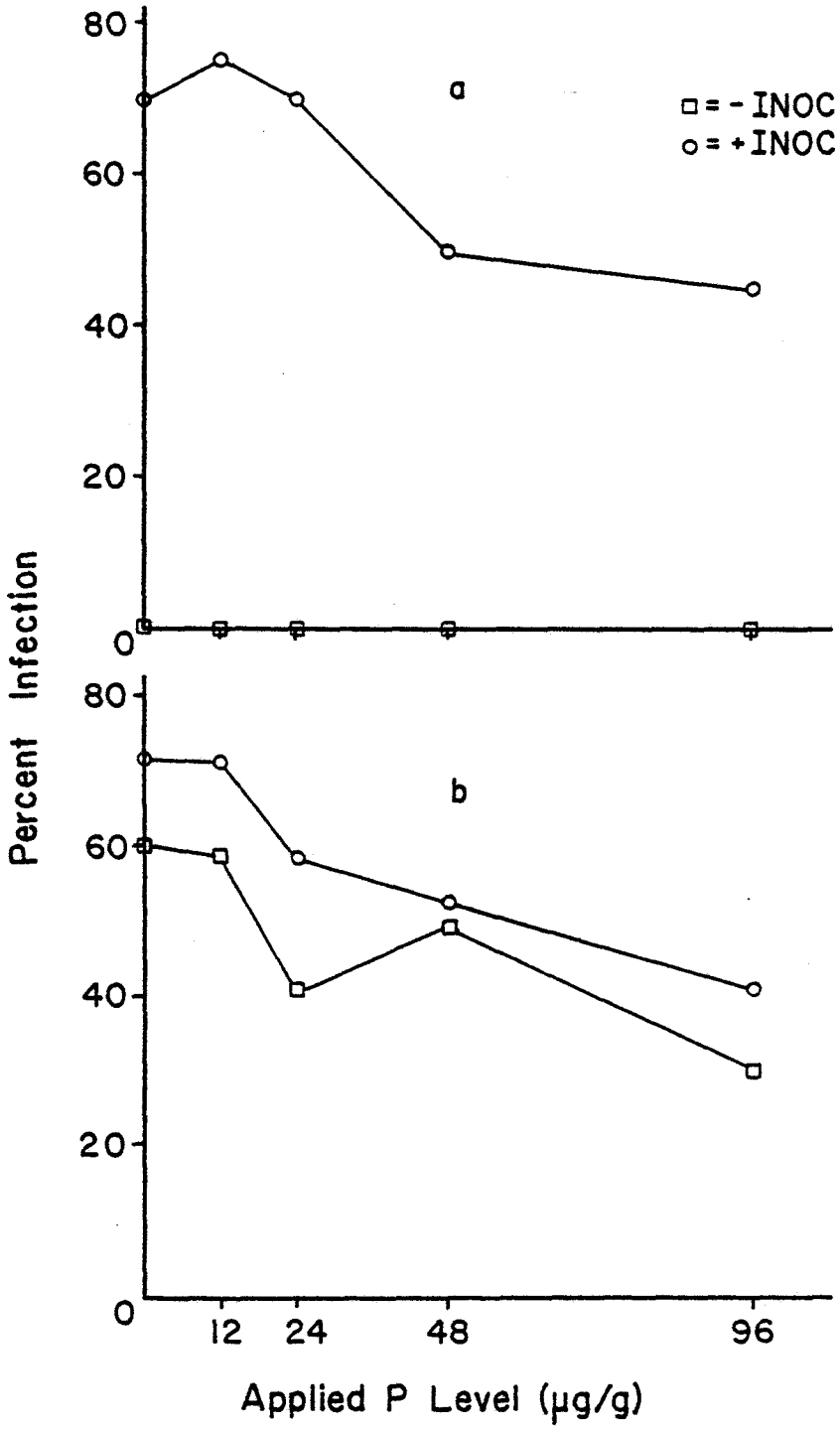


Figure 3: Effect of applied phosphate on mycorrhizal infection in roots of cowpea plants grown in a) fumigated soil and b) non-fumigated soil.

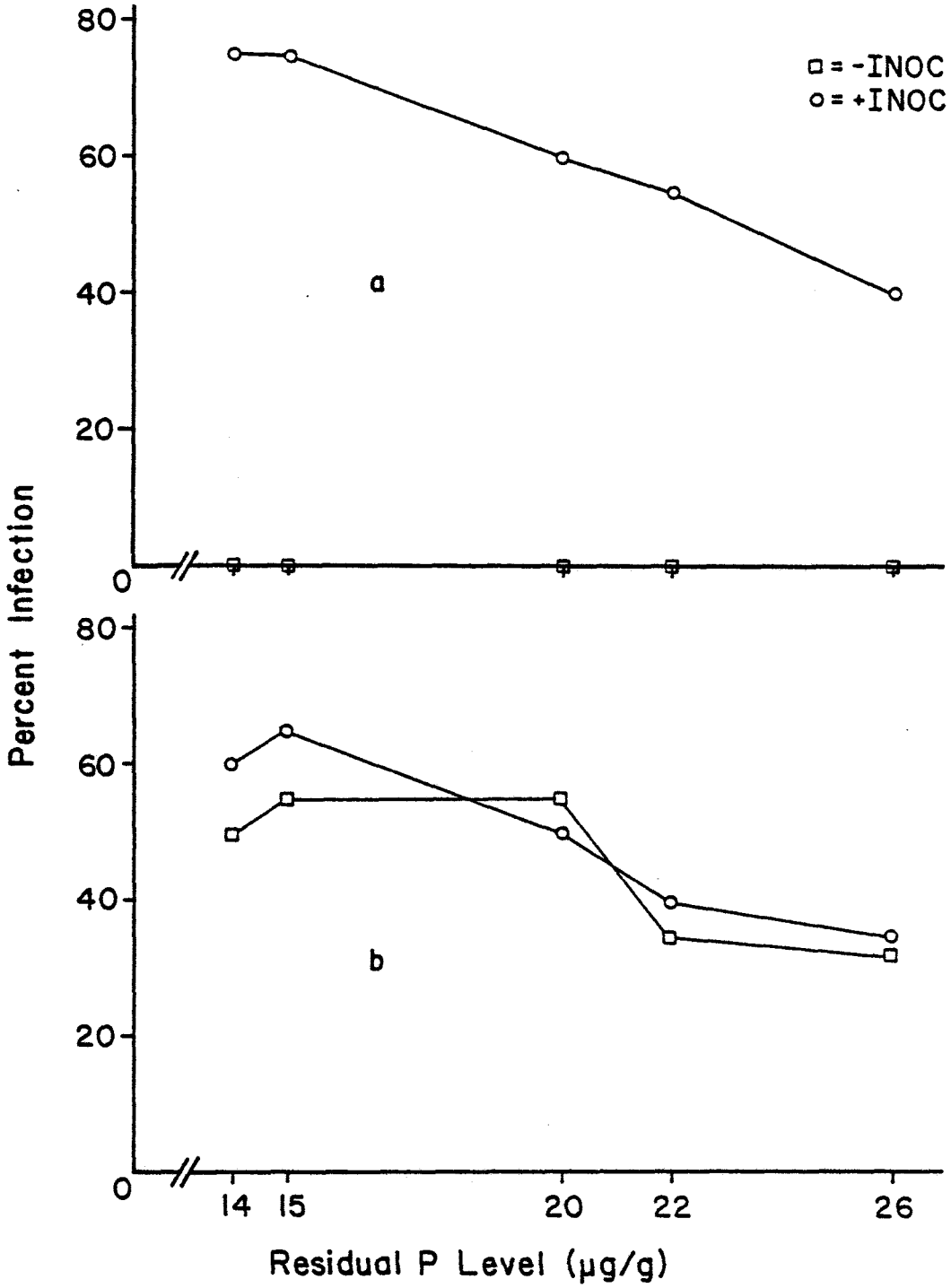


Figure 4: Effect of residual soil phosphate on mycorrhizal infection in roots of cowpea plants grown in a) fumigated soil and b) non-fumigated soil.

Table 6 :Effect of mycorrhizal inoculation on growth of Cowpea plants in fumigated and non-fumigated soils (means of 3 replicates).

| Soil P Treatment | Fumigation | Inoculation | Growth Variables HT1 cm | HT2 cm | HT3 cm | RTW g/plant | STW g/plant | POD no/plant | YLD g/plant | NOD no/plant | INF % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Applied P | - | - | 32.1 | - | - | 4.1 | 15.5 | 4.3 | 3.9 | 6.8 | 46.2 |
| | | + | 32.8 | - | - | 4.6 | 15.7 | 4.0 | 4.6 | 6.9 | 55.6 |
| | + | - | 16.5 | 18.4 | 18.4 | 2.0 | 1.6 | 0.4 | 0.4 | 3.4 | 0 |
| | | + | 16.9 | 23.7 | 35.9 | 2.5 | 4.5 | 2.6 | 2.7 | 4.8 | 62.9 |
| Residual P | - | - | 28.8 | - | - | 9.0 | 5.5 | 2.7 | 2.3 | 5.5 | 45.3 |
| | | + | 29.9 | - | - | 9.3 | 6.4 | 3.1 | 2.3 | 5.8 | 49.7 |
| | + | - | 16.5 | 15.1 | 15.6 | 1.7 | 1.8 | 0 | 0 | 3.1 | 0 |
| | | + | 17.5 | 22.8 | 27.5 | 2.8 | 2.5 | 2.6 | 1.9 | 4.9 | 62.1 |

HT1, HT2, HT3 = Height at 30, 60 and 90 days respectively. RTW = Root weight, STW = Stover weight, POD = Pod yield, YLD = Seed yield, INF = percent infection, NOD = Nodulation rating (see text for rating method).

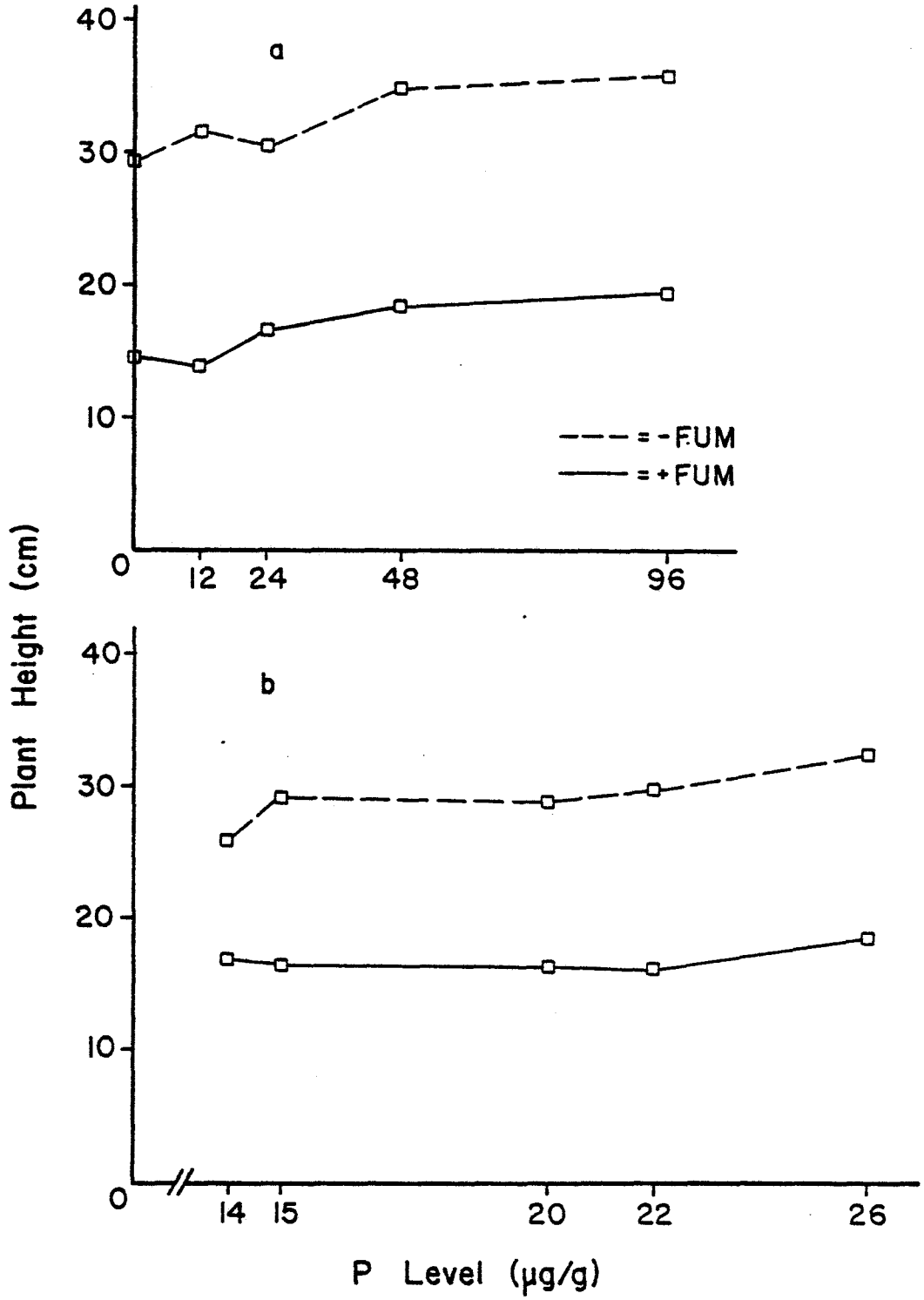


Figure 5: Effect of methyl bromide fumigation and P level on cowpea plants grown for 30 days in soils with a) newly applied P and b) residual soil P.

in height of the same plant on the 3 dates height measurements were taken. Certain plants became shorter after 60 days than after 30 days because their tips withered and no more leaves were formed (Table 7 and 8). Height measurements were taken from ground level to the tip of the tallest leaf.

Mosse and Hayman (1971) also reported that noninoculated onion seedlings became shorter after 2 weeks of growth because their tips withered and this persisted up to harvest. Growth depressions due to methy bromide fumigation have also been reported by Yost and Fox (1979) who found that some of the 6 species studied did not progress beyond the seedling stage in fumigated soils. Kormanik et al. (1977) also found that non-mycorrhizal sweetgum seedlings failed to exceed 5 cm in height in sterilized soil regardless of soil fertility. Similar deleterious effects of methyl bromide fumigation have been reported by Rovira (1976) and Martin et al. (1963). These effects may be attributed to direct toxic conditions in the soil that sometimes follow soil sterilization. For example, increase in Mn, Al and phytotoxic residues in soil have been reported when soil was treated with bromide-containing fumigants such as methyl bromide and ethylene dibromide (Kreutzer, 1960; Aldrich and Martin, 1952). As will be seen later, in this experiment, large quantities of Mn and Al were released into the soil following fumigation. There is evidence in the literature

Table 7: Growth of Cowpea plants inoculated with Glomus mosseae at 5 levels of applied phosphate in fumigated soil (means of 3 replicates)

| Applied P Level µg/g | Inoculation | Growth Variables HT1 cm | HT2 cm | HT3 cm | RTW g/ plant | STW g/ plant | POD no/ plant | YLD g/ plant | NOD no/ plant | INF % |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | - | 20.7 | 15.7 | 14.9 | 1.8 | 6.5 | 0.8 | 0.9 | 3.5 | 20.8 |
| | + | 21.7 | 19.7 | 26.7 | 2.4 | 7.6 | 2.0 | 2.2 | 4.0 | 60.2 |
| 12 | - | 21.3 | 15.1 | 16.0 | 2.8 | 6.9 | 1.3 | 1.3 | 4.1 | 18.9 |
| | + | 22.4 | 20.1 | 29.9 | 3.6 | 9.7 | 2.5 | 2.5 | 5.3 | 71.7 |
| 24 | - | 22.3 | 18.4 | 18.2 | 3.3 | 6.9 | 1.5 | 1.3 | 4.8 | 13.9 |
| | + | 22.9 | 25.0 | 43.2 | 3.8 | 0.2 | 3.0 | 3.5 | 6.3 | 65.2 |
| 48 | - | 25.3 | 21.2 | 19.7 | 3.5 | 7.2 | 1.8 | 1.3 | 6.4 | 15.9 |
| | + | 25.6 | 27.5 | 40.2 | 3.7 | 9.1 | 3.5 | 3.8 | 6.8 | 49.7 |
| 96 | - | 26.3 | 21.9 | 23.4 | 3.9 | 8.5 | 3.0 | 2.7 | 6.7 | 9.9 |
| | + | 25.9 | 26.3 | 39.5 | 4.2 | 9.6 | 4.2 | 4.6 | 7.0 | 42.6 |

HT1, HT2, HT3 = Height at 30, 60 and 90 days respectively. RTW = Root weight, STW = Stover weight, POD = Pod yield, YLD = Seed yield, INF = percent infection, NOD = Nodulation rating (see text for rating method).

Table 8: Growth of cowpea plants inoculated with Glomus mosseae at 5 levels of residual soil P in fumigated soil (means of 3 replicates).

| P level* µg/g | Inoculation | Growth Variables | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | HT1 cm | HT2 cm | HT3 cm | RTW g/ plant | STW g/ plant | POD no/ plant | YLD g/ plant | NOD no/ plant | INF % |
| 14 | - | 19.8 | 15.4 | 15.1 | 4.7 | 2.9 | 0.8 | 0.8 | 3.5 | 16.9 |
| | + | 21.9 | 20.5 | 21.4 | 5.2 | 3.8 | 2.3 | 1.8 | 4.1 | 70.3 |
| 15 | - | 21.2 | 13.6 | 13.7 | 5.3 | 3.2 | 0.8 | 0.5 | 3.5 | 18.9 |
| | + | 22.9 | 21.2 | 25.8 | 6.2 | 3.9 | 2.2 | 1.7 | 4.4 | 71.7 |
| 20 | - | 21.3 | 14.9 | 15.7 | 5.3 | 3.7 | 0.8 | 0.7 | 3.9 | 18.9 |
| | + | 22.6 | 22.6 | 30.9 | 6.2 | 4.3 | 3.0 | 1.9 | 5.3 | 56.5 |
| 22 | - | 21.7 | 14.8 | 14.9 | 5.3 | 3.8 | 1.0 | 1.0 | 5.1 | 11.9 |
| | + | 22.4 | 20.5 | 29.7 | 5.9 | 4.6 | 2.8 | 2.3 | 6.1 | 50.6 |
| 26 | - | 24.9 | 16.8 | 18.9 | 5.9 | 4.6 | 2.3 | 1.8 | 5.5 | 10.9 |
| | + | 24.2 | 21.2 | 29.8 | 6.5 | 5.5 | 3.5 | 2.7 | 6.6 | 38.0 |

HT1, HT2, HT3 = Height at 30, 60 and 90 days respectively. RTW = Root weight, STW = Stover weight, POD = Pod yield, YLD = Seed yield, INF = percent infection, NOD = Nodulation rating (see text for rating method).

* Residual P level as measured by the modified Truog extractant.

which indicates that the rhizosphere microflora can bind Mn thus making it unavailable to plants (Gerretsen, 1937). It is believed that "the treatment of the soil with volatile biocides temporarily increases the quantity of minor elements by killing microorganisms capable of binding these elements (Kreutzer, 1960). On the other hand, Martin and co-workers (1953) reported that Mn deficiency of citrus was overcome by treatment of the soil with ethylene dibromide.

Another unfavorable result of fumigation is that of disease accentuation and disease exchange collectively termed the "boomerang phenomenon" by Kreutzer (1960). Disease accentuation can occur when soil is treated to control soil organisms. This may result because of the killing or inhibiting of enemies or competitors that normally keep soil-borne pathogens in check thus permitting an invading pathogen to grow through the soil without biological opposition (Altman, 1970; William and Salt, 1970). Fumigation may also have beneficial effects on plant growth. Results of research from many workers indicate that fumigation of the soil often results in increased plant growth (Altman, 1970; Rovira, 1976; Kleinschmidt and Gerdemann, 1972). This increased growth following fumigation is attributed to a combination of factors including a) enhanced release of $NH_4^+$-N and nitrogenous compounds from the soil with a consequent increase

in N uptake (Rovira, 1976; Altman and Tsue, 1965), b) reduction of root pathogens (Yost and Fox, 1979) and c) alteration of the soil microflora including enhancement of some beneficial microorganisms, for example, Altman and Tsue (1965) isolated Pseudomonas from fumigated soil that was capable of stimulating plant growth.

Effects on Nodulation

Nodulation was found to be lower in fumigated than in non-fumigated soil and inoculation with VA mycorrhiza increased nodulation in both fumigated and non-fumigated soils. The increase was greater, however, in the fumigated soil especially at the low rate of P (Figure 6 and 7). Yost and Fox (1982) have suggested that residual Br left in the soil following fumigation may adversely affect rhizobia and thus reduce nodulation or that rhizobium inoculum alone in fumigated soil may not be as effective as inoculum plus indigenous rhizobia in non-fumigated soil.

Effects on Nutrient Uptake

The fumigated soil contained considerably greater quantities of Al and Mn than the non-fumigated soil as evident from plant tissue analysis (Table 9 and 10). It is interesting to note that the non-inoculated (non-mycorrhizal) plants in the fumigated treatments took up 142% more Al and 428% more Mn than the inoculated plants

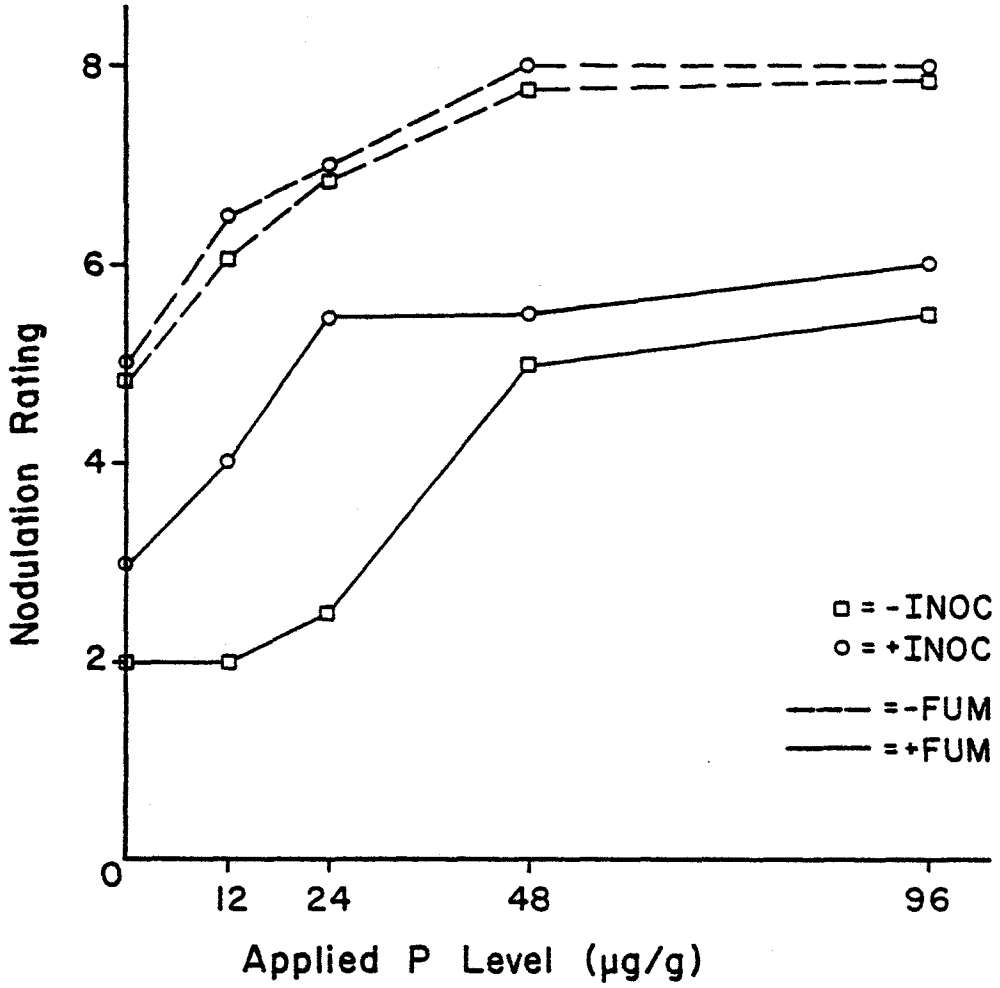


Figure 6: Nodulation rating of cowpea plants inoculated with Rhizobium and grown at 5 levels of applied phosphate in fumigated and non-fumigated soils with and without inoculation with VA mycorrhiza.

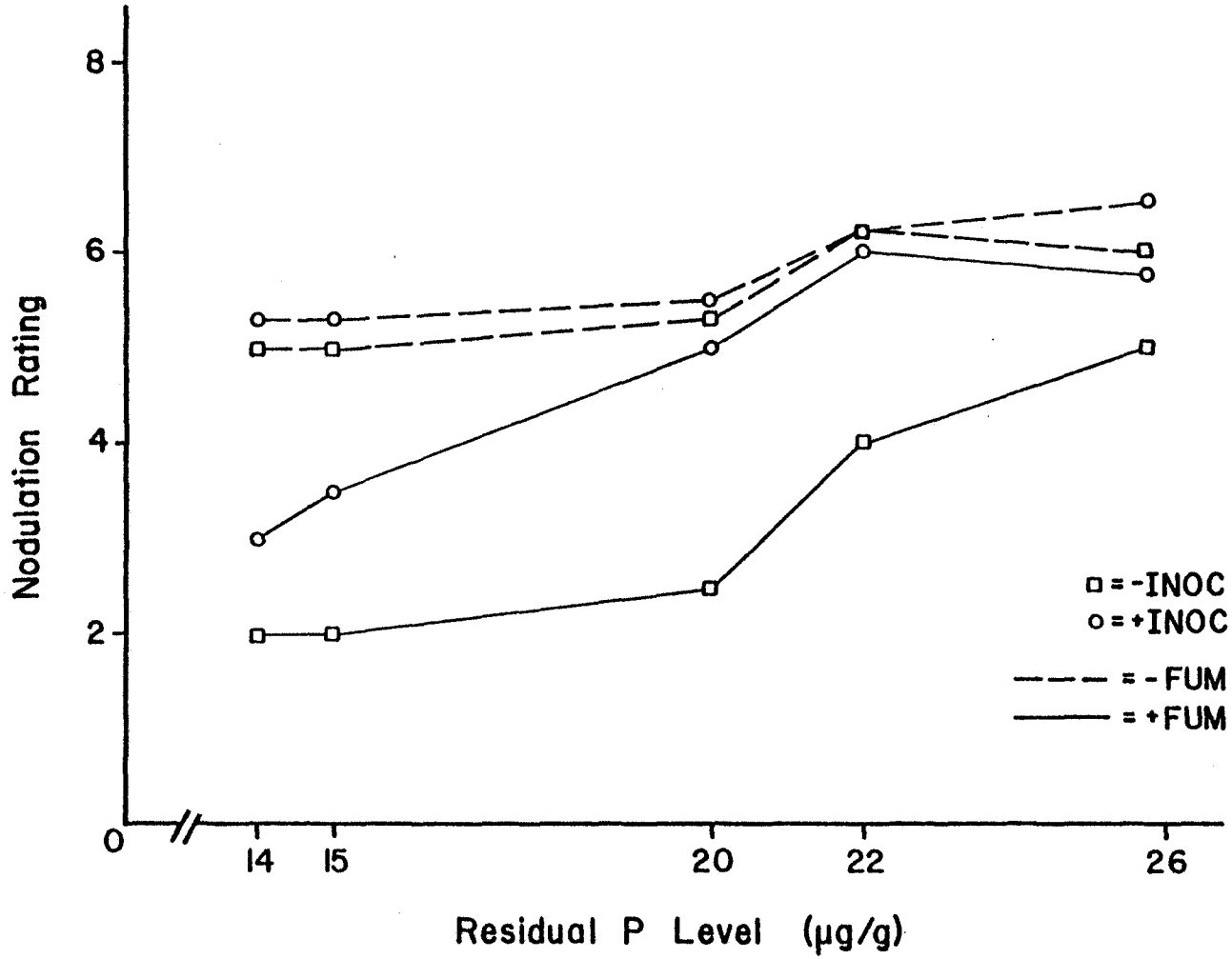


Figure 7: Nodulation rating of cowpea plants inoculated with Rhizobium and grown at 5 levels of residual phosphate in fumigated and non-fumigated soils with and without inoculation with VA mycorrhiza.

Table 9: Effects of methyl bromide fumigation on nutrient concentration in leaves of mycorrhizal and non-mycorrhizal cowpea plants sampled at 50% flowering and grown in fumigated and non-fumigated soils with freshly applied phosphate (means of 3 replicates).

| Fumigation | Inoculation | N | P | K | Ca | Mg | S | Si | Na | Cl | Al | Mn | Fe | Cu | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Percent | | | | | | | | | ppm | | | | |
| - | - | 2.65 | 0.13 | 2.06 | 1.83 | 0.24 | 0.15 | 0.14 | 0.08 | 0.63 | 859 | 373 | 99 | 5.3 | 54.4 |
| | + | 2.56 | 0.13 | 1.79 | 1.55 | 0.24 | 0.14 | 0.12 | 0.08 | 0.55 | 1337 | 335 | 96 | 8.7 | 42.90 |
| + | - | 4.50 | 0.15 | 2.82 | 1.59 | 0.57 | 0.19 | 0.16 | 0.09 | 0.36 | 3234 | 1768 | 108 | 5.0 | 49.00 |
| | + | 4.60 | 0.20 | 2.76 | 1.71 | 0.36 | 0.19 | 0.17 | 0.09 | 0.42 | 4565 | 906 | 127 | 4.9 | 60.00 |

Table 10: Effects of methyl bromide fumigation on nutrient concentration in leaves of mycorrhizal and non-mycorrhizal cowpea plants sampled at 50% flowering and grown in fumigated and non-fumigated soil with residual phosphate (means of 3 replicates).

| Fumigation | Inoculation | N | P | K | Ca | Mg | S | Si | Na | Cl | Al | Mn | Fe | Cu | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Percent | | | | | | | | | ppm | | | | |
| - | - | 2.43 | 0.12 | 1.86 | 1.52 | 0.29 | 0.14 | 0.14 | 0.08 | 0.59 | 902 | 299 | 98 | 4.9 | 49 |
| | + | 2.48 | 0.13 | 1.93 | 1.75 | 0.35 | 0.15 | 0.16 | 0.08 | 0.65 | 603 | 301 | 100 | 5.2 | 55.3 |
| + | - | 2.73 | 0.11 | 1.87 | 1.19 | 0.32 | 0.11 | 0.15 | 0.08 | 0.47 | 4554 | 943 | 94 | 1.0 | 60 |
| | + | 3.89 | 0.18 | 2.64 | 1.72 | 0.36 | 0.17 | 0.15 | 0.08 | 0.43 | 4393 | 408 | 106 | 7.0 | 69 |

in the non-fumigated treatments, i.e., 3234 ppm compared to 1337 ppm Al and 1768 ppm compared to 335 ppm Mn respectively (Table 9). This might have caused Al and Mn toxicity and hence depressed the growth of non-inoculated plants growing in the fumigated soil. The fact that growth of inoculated plants in fumigated soil was not as severely depressed suggested that mycorrhiza may be involved in overcoming mineral toxicity in plants particularly Al and Mn. This needs to be confirmed, however.

Another factor which should be considered is that in using the X-Ray Fluorescence Quantimeter for nutrient analysis of plant tissue, the peak for Al and Br nearly coincide (Okazaki, personal communication).* It is therefore possible that the increase in Al concentration in the leaves of plants growing in the fumigated soil may only be apparent and that the peak measured may have been that of Br plus Al. However, it was also found that the inoculated plants in non-fumigated soil contained more Al in their leaves than the non-inoculated plants (Table 14 and 16). If the assumption about Br interference in Al analysis is correct, then this may mean that inoculated plants also took up more Br than Al. If this implied effect of bromide is not true, then it appears that

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*E. N. Okazaki--Dept. of Agronomy and Soil Science, Univ. of Hawaii.

inoculated plants take up more Al than non-inoculated plants.

Leaf N concentration increased significantly ($P < 0.05$) with fumigation as did leaf composition of Al and Mn in both the soil with newly applied and residual P (Appendix Table 30). These data differ from those of Yost and Fox (1982) who found that fumigation had little effect on N content of leaves of cowpea.

Leaf concentration of P, K, Mg, Si, Cl and Fe also increased significantly ($P < 0.05$) with fumigation in both soils. Concentration of S in leaves was significantly increased by fumigation only in the soil with newly applied P while Zn values increased significantly only in the soil with residual P (Appendix Table 30). Furthermore, decreases in soil pH and consequent increase in exchangeable Al and Al saturation following methyl bromide fumigation have been reported (Lopes and Wollum, 1976). This may contribute to decreased growth of plants. In the present experiment, although pH of the soil was adjusted to 5.5 at the beginning of the experiment, soil pH from some selected treatments decreased by 0.2 units in the low P level. In the high P treatments, pH was found to have increased beyond that to which the soil had been limed (from pH 5.5 to 6.0). This may be due to the fact that monocalcium phosphate used to supply P may have contributed to the pH increase.

## Effects of Mycorrhizal Inoculation

### Effects on Growth and Yield

Growth response curves for plant height for inoculated and non-inoculated plants in sterilized soil are presented in Figures 8 and 9. The mycorrhizal (inoculated) plants grew better than the non-mycorrhizal ones at all levels of applied and residual P and the difference became greater with time. There was no significant difference between inoculated and non-inoculated plants during the first month in both soils. Lack of difference between them at this stage may be attributed to the fact that inoculated plants had not been infected for sufficient time to produce measurable differences. Moreover, mycorrhiza may take several weeks to develop and during this development period, it may be parasitic, receiving organic nutrients from the host and benefiting it little or not at all until it has devleoped external mycelia in the soil (Lindermann and Hendrix, 1982). Additionally, during the early stages of growth, autotrophic plants depend more on reserves for their nutrition than on nutrients absorbed from the soil until their root systems have developed.

By the end of the second and third months of growth, inoculated plants had grown much taller than non-inoculated ones at all levels of P in both soils. Mycorrhizal plants at the lowest P level grew better than non-mycorrhizal plants at the highest P level (Figures 8 and 9 at 60 and

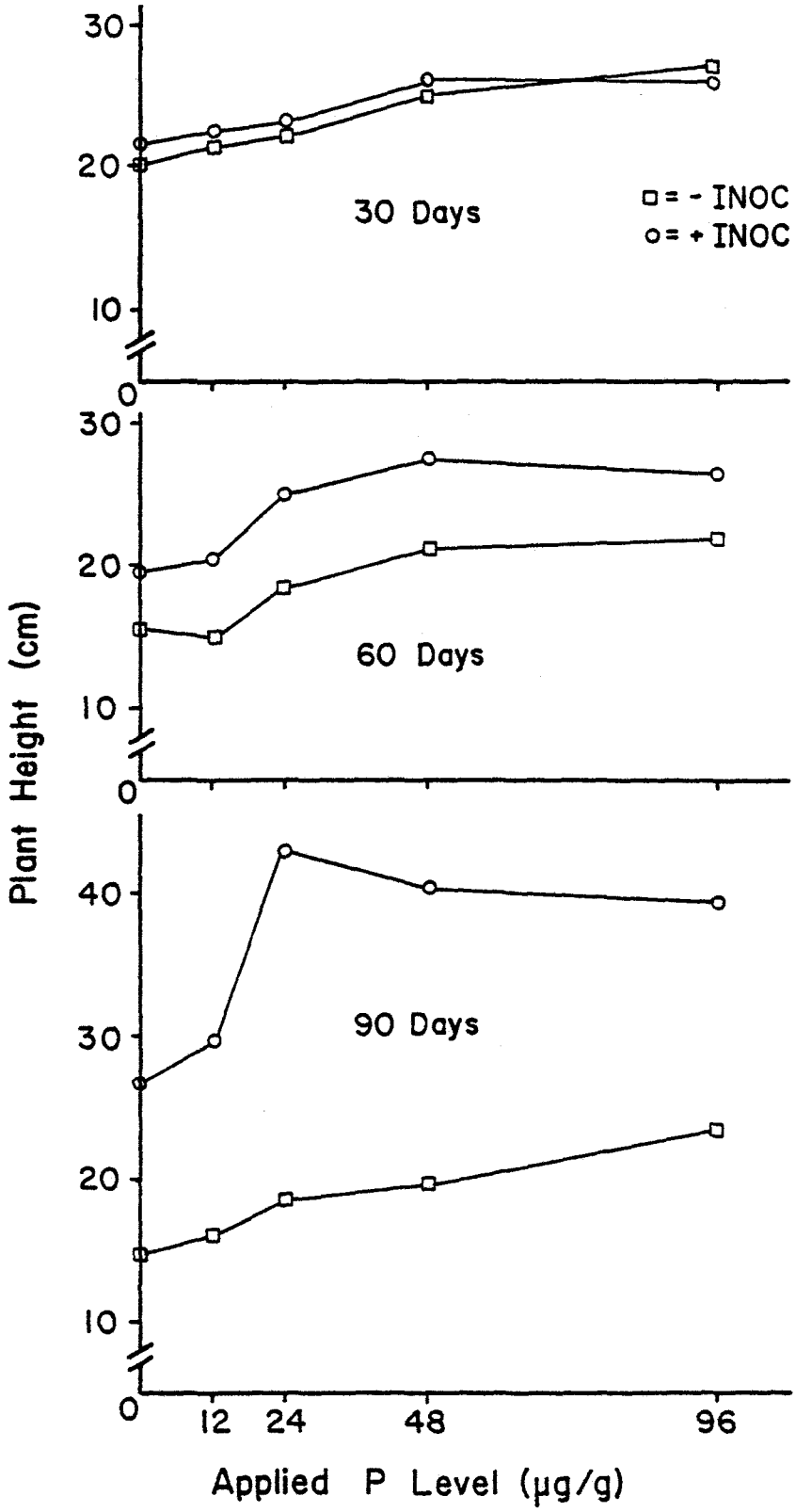


Figure 8: Effect of inoculation with Glomus mosseae and applied phosphate on growth of cowpea plants at 3 ages in fumigated soil.

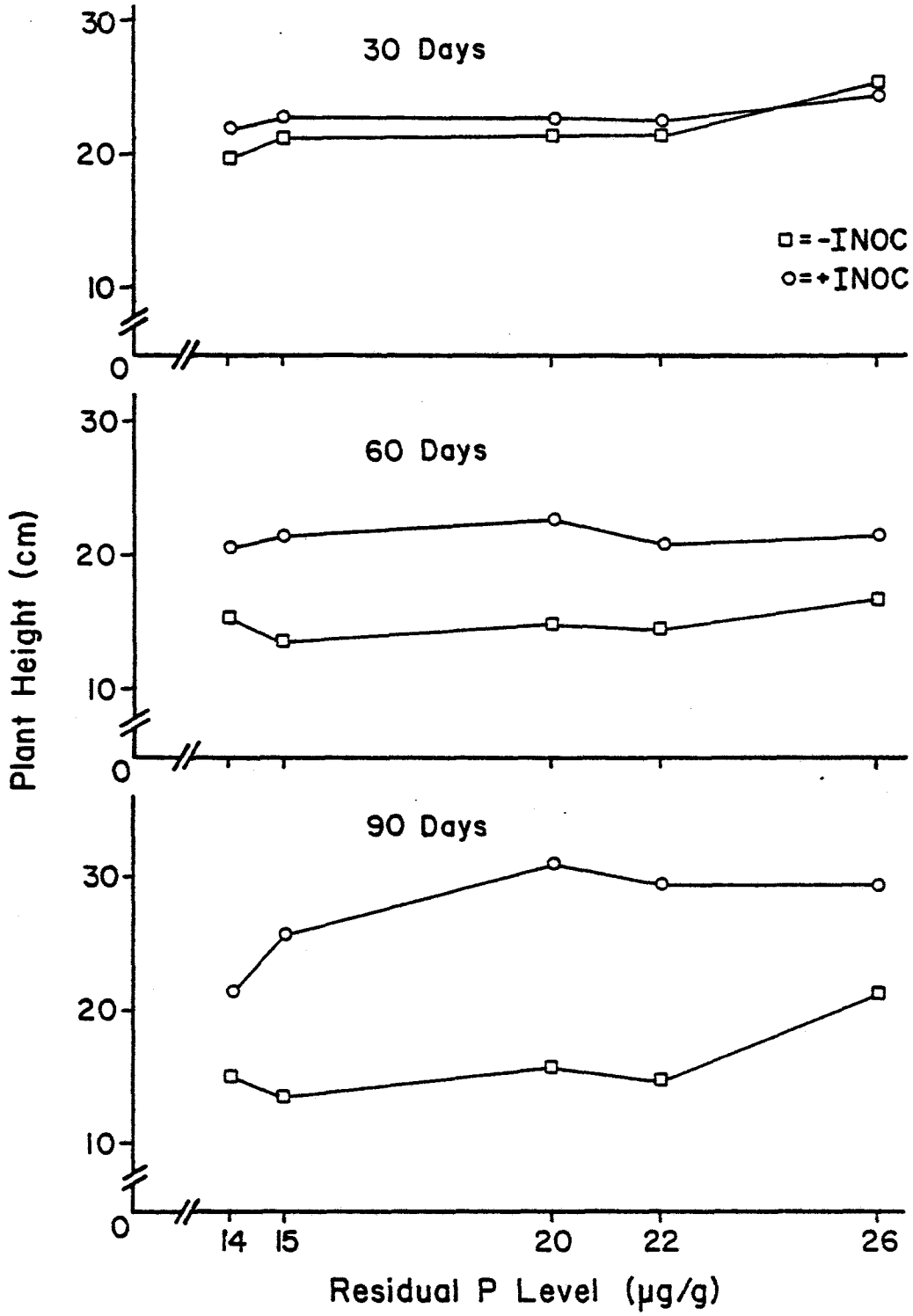


Figure 9: Effect of inoculation with Glomus mosseae and residual phosphate on growth of cowpea plants at 3 ages in fumigated soil.

90 days), thereby confirming the fact that mycorrhizal contribution to plant growth is greatest when soil P is low. The biggest increase occurred at the intermediate P levels, viz; 24 µg P/g for the soil with newly applied P and 20 µg P/g for the soil with residual P (Figures 8 and 9 at 90 days). At the highest P level, growth of the inoculated plants tended to level off in both soils while that of the non-inoculated increased steadily.

Growth response of roots to inoculation was less than that of tops (Tables 7 and 8). Although inoculation with VA mycorrhiza increased root growth over the controls, the differences were not significant in the soil with newly applied P. They were significant ($P < 0.01$) in the soil with residual P (Appendix Table 27). Pod and seed yield were significantly increased by inoculation at the 1% level in both soils (Appendix Table 27).

The most important function of mycorrhiza is its improvement of plant growth through the enhancement of P uptake. This is achieved by making use of the absorbing capacity of the extensive network of hyphae usually associated with an infected root (Gerdemann, 1975; Tinker, 1975). This hyphal network increases the effective volume of soil from which P can be absorbed by exploring the soil beyond the depletion zone which is usually developed around roots, particularly for nutrients that diffuse slowly in the soil, notably phosphorus. Also research by many workers has

shown that mycorrhiza greatly improve growth of plants supplied with insoluble and less readily available phosphate sources such as bonemeal, rockphosphate or apatite (Daft and Nicolson, 1966; Murdoch et al., 1967). The extra P is known to be taken up from the soluble fraction associated with such sources or released from them in the soil (Mosse, 1977b). Mycorrhizae therefore ensure more efficient utilization of the available phosphate rather than the solubilization of the less soluble forms as was suggested by Hattingh and his co-workers (1973).

Ultimately we are interested in knowing to what extent mycorrhiza can be used to substitute for phosphate fertilizers or at what level of applied or residual soil P does the mycorrhizal effect disappear. The answer to these questions depend on several factors including adequacy and/or efficiency of mycorrhizal fungi found in any soil, nutrient status of the soil, responsiveness of the host plant to mycorrhizal infections and other factors of the soil-root environment such as pH, biological activity, etc. In a typical mycorrhizal response, mycorrhizal plants growing at low levels of soil fertility usually grow better and take up more nutrients especially P than non-mycorrhizal plants. At high levels of soil P, non mycorrhizal plants grow as well as or better than mycorrhizal plants (Bayis, 1967; Daft and Nicolson, 1966).

This is because when soil P is adequate plants can take up their requirement of P with the aid of their roots alone. By contrast, mycorrhizal plants growing in high P situations will tend to accumulate supra-optimal amounts of P which may lead to toxicity and growth depression (Mosse, 1973b).

It was not possible in this experiment to determine at what level of soil P plants may be expected to cease benefiting from mycorrhizal infections. Fumigation depressed the growth of the non-inoculated treatments almost to the extent of eliminating the growth response expected at high P levels (Figures 8 and 9). What seemed to be happening was that the inoculated plants were better able to withstand the adverse effects of fumigation. Consequently, they grew to maturity and yielded pods while the non-inoculated plants remained stunted and lost all their leaves except at the highest P levels, as noted earlier. The potential of mycorrhiza to promote plant growth thus seems to be related to its ability to alleviate some physical constraints to the acquisition of nutrients from soil by plants.

Results of pod and seed yield in sterilized and unsterilized soils are presented in Figures 10 and 11. As was the case with plant height (Figures 8 and 9 at 60 and 90 days), mycorrhizal plants growing in sterilized soil at the lowest P level yielded better than non-

mycorrhizal plants at one of the highest P level (48 µg P/g). This may indicate the possible savings on phosphate fertilizers that can be obtained by pre-inoculating plants with effective strains of VA mycorrhiza in P-deficient soil. For example, in the fumigated soil pod yield was 3 pods/plant (Figure 10) and seed yield was 2.9 g/plant (Figure 11) at the highest P level when the plants were not inoculated with VA mycorrhiza. The same pod yield (3 pods/plant) and a slightly higher seed yield (3.1 g/plant) were obtained in the non-fumigated soil at the lowest P level when the plants were inoculated with VA mycorrhiza. Pod and seed yield ranged from 3.5 - 4.5 pods/plant and 3.7 - 5 g/plant, respectively in non-fumigated soils at 12 and 24 µg P/g with either inoculation or non-inoculation, but the increase resulting from inoculation was generally higher for seed yield. Although extrapolation of results from greenhouse experiments to field situations cannot be done with confidence, these data at least give an indication that mycorrhiza may be substituted for P fertilizer, especially in low P situations. Menge et al. (1978) estimated that it may be possible to save from $45-$226/acre in cost of fertilizers by inoculating citrus grown in fumigated soil with mycorrhizal fungi.

Since native mycorrhiza were present in the non-fumigated soil, differences between inoculated and non-

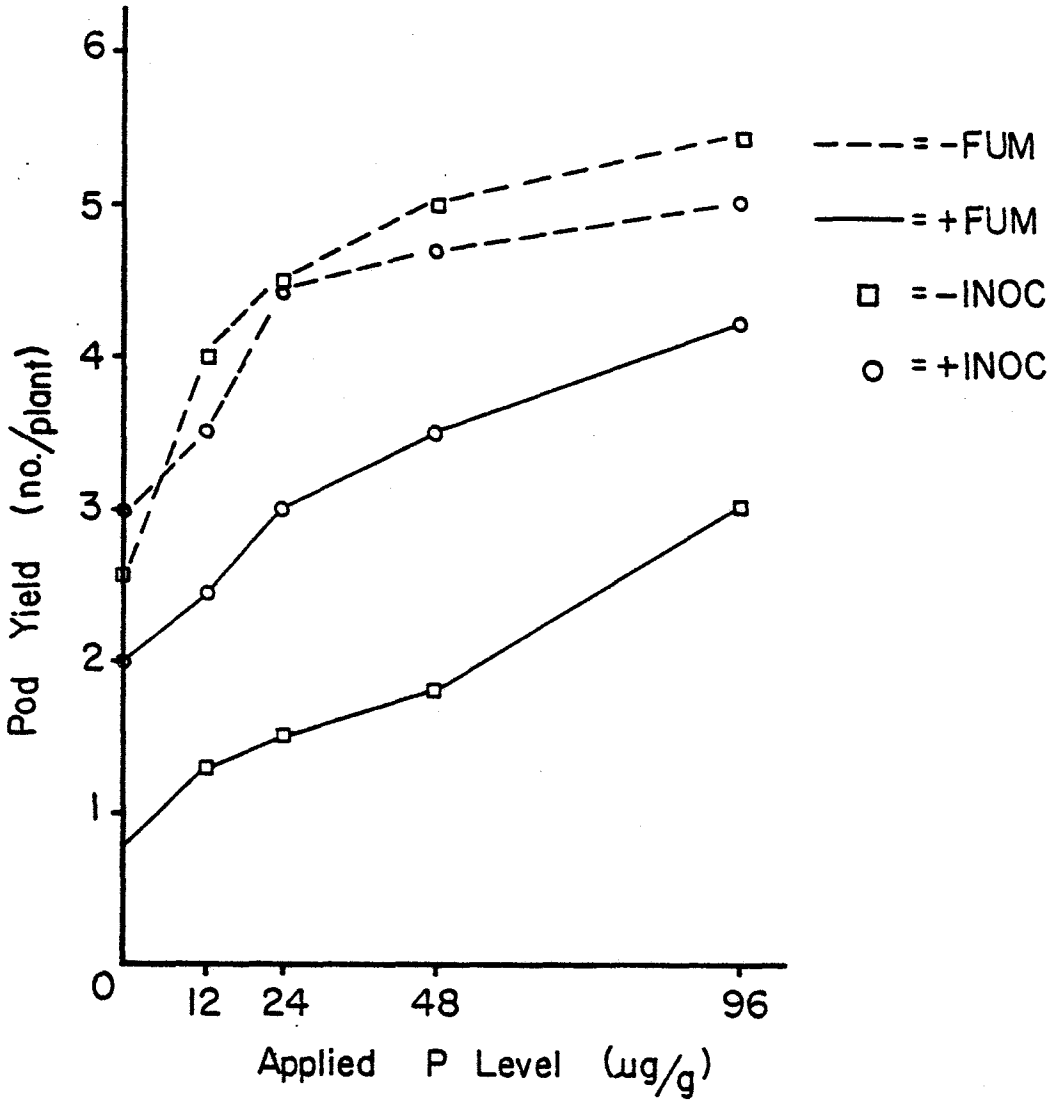


Figure 10: Effect of inoculation with Glomus mosseae on pod yield of cowpea grown in fumigated and non-fumigated soils at 5 levels of applied phosphate.

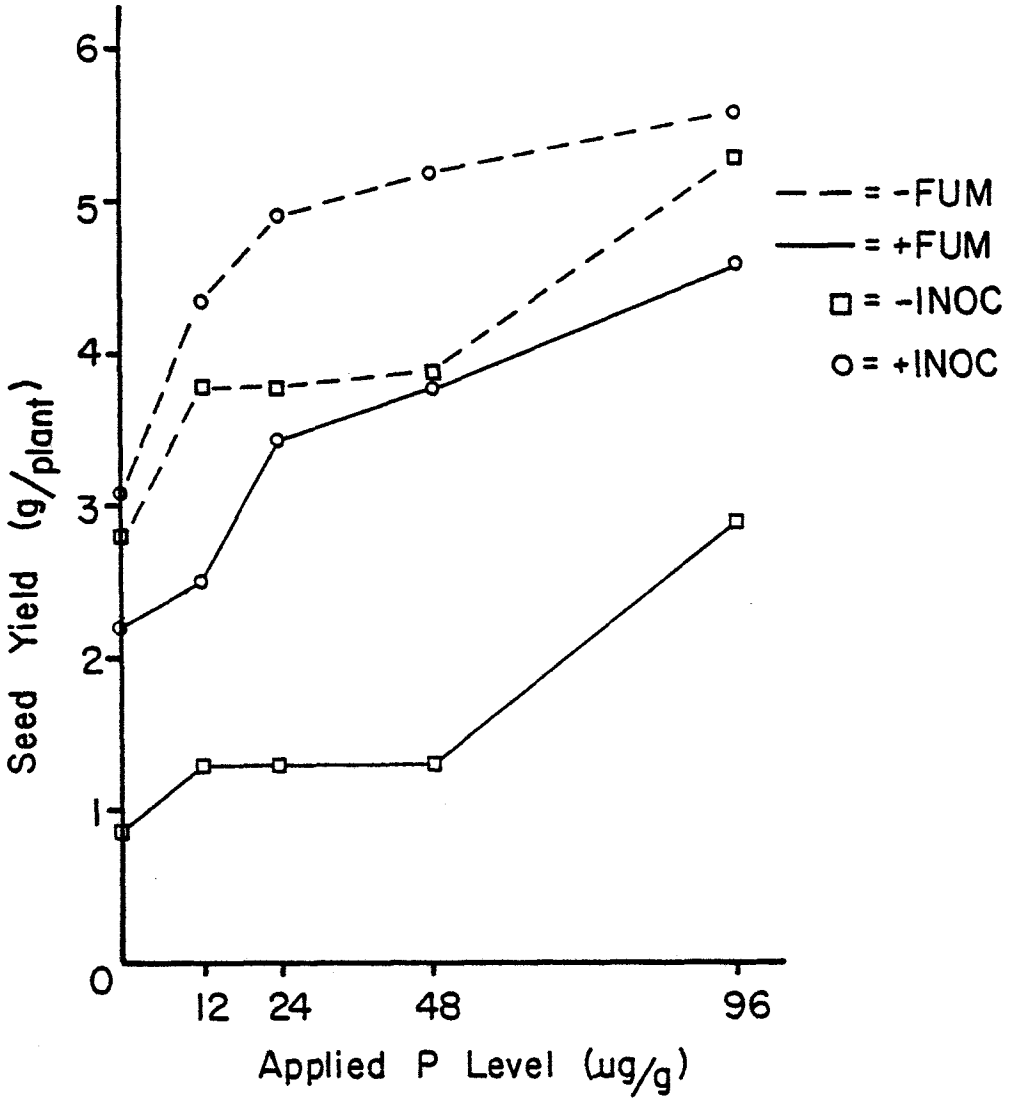


Figure 11: Effect of inoculation with Glomus mosseae on seed yield of cowpea grown in fumigated and non-fumigated soils at 5 levels of residual phosphate.

inoculated treatments were expected to be small. This proved to be the case under the experimental conditions (Figures 10 and 11). If inoculation with VA mycorrhiza in unsterilized soil is to be beneficial to the plant, it must be known to be more efficient than the indigenous population. In this experiment, it may be assumed that the introduced mycorrhiza had a competitive advantage over the indigenous mycorrhizal population or were more effective since the growth variables were increased by inoculation in the unsterilized soil. Mosse (1975) has provided evidence that non-indigenous mycorrhiza can be introduced into both sterilized and unsterilized soil and that these can become established in competition with the native population and can improve plant growth. Similar results have been reported by Powell (1977). Introduced mycorrhiza may also be less effective than indigenous species (Powell and Sithamparanathan, 1977).

The practical application of field inoculation however, is seriously limited by the inability to culture mycorrhiza artificially and by the fact that little is known about the persistence and rate of spread of an introduced mycorrhiza in the soil (Mosse, 1975). The observation of Kleinschmidt and Gerdemann (1972) in fumigated citrus nurseries that patches of healthy plants inoculated with VA mycorrhiza were found scattered through out the nursery may suggest that introduced mycorrhiza

can spread fairly well in the soil. The extent of such spread in unsterilized soil is not known but is most likely to be much less due to competition with native mycorrhiza.

Effects on Root Infection

Figure 3 and 4 show that roots of plants in all treatments in the non-fumigated soil were infected with VA mycorrhiza whether the plants were inocualted or not. Well developed external hyphae and vesicles were observed in all plants in the non-fumigated soil. Infection was however higher in the inoculated treatments. In the fumigated soil, only the inoculated treatments were infected by mycorrhiza. Comparison of infection in inoculated plants in fumigated and non-fumigated soils showed that infection was generally higher in the fumigated than in the non-fumigated soil. Regression analysis of percent root infection and plant height at final harvest showed that there was a highly significant relationship between extent of root infection and growth (Figure 12 and 13). Correlation coefficients of 0.712 and 0.693 were obtained for the soil with newly applied and residual P respectively.

Infection was also found to decrease as the level of applied and residual soil phosphate increased. The decrease was greater in the soil with newly applied P (Figures 3 and 4).

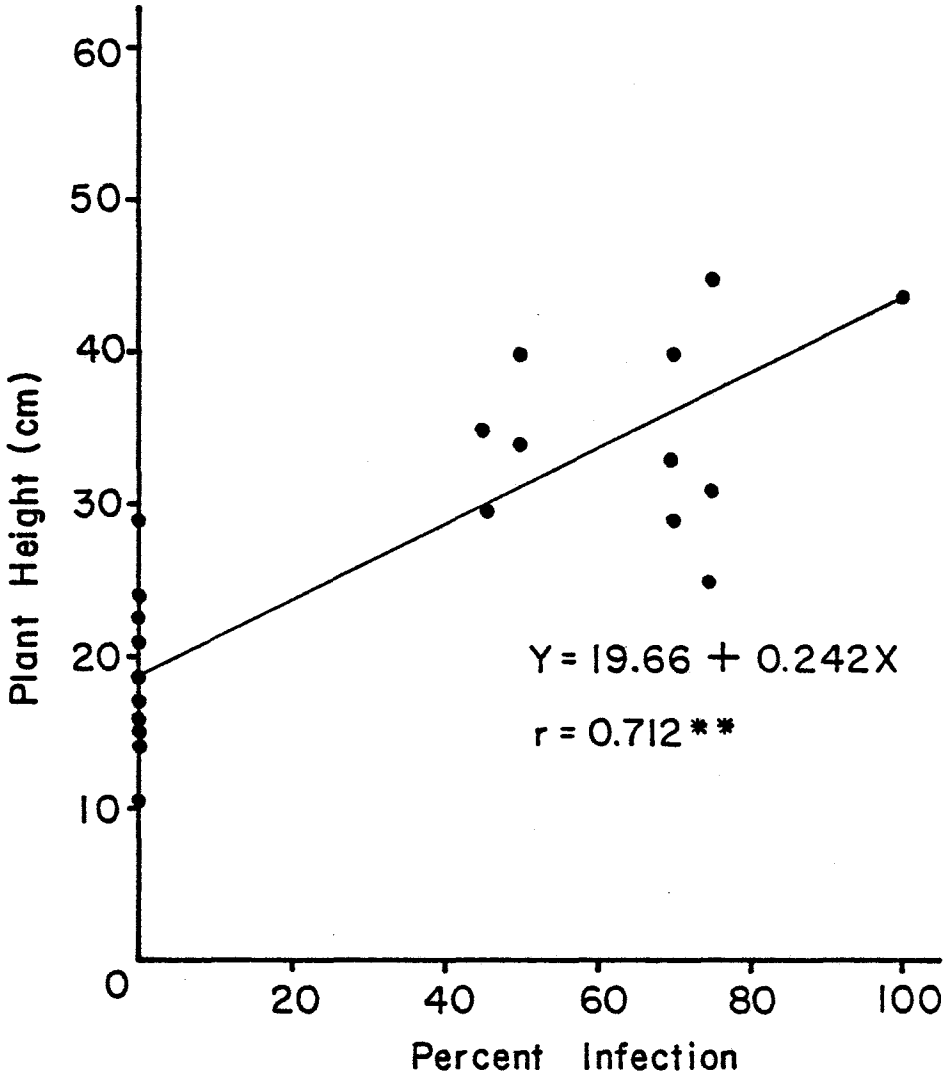


Figure 12: Relationship between percent mycorrhizal infection in roots of cowpea plants and plant height after 90 days of growth in fumigated soil with applied P.

The extent to which plants depend on mycorrhiza for improved growth is dictated by the level of soil fertility.

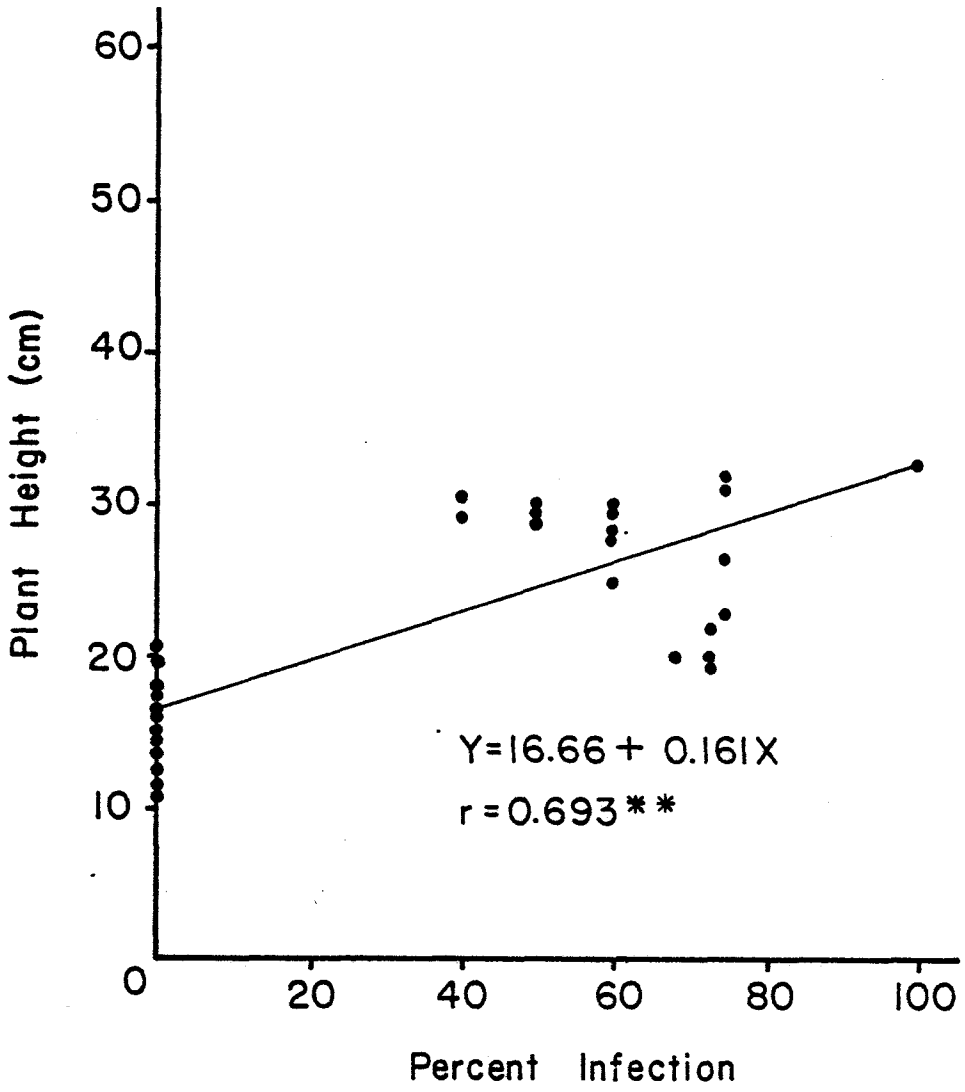


Figure 13: Relationship between percent mycorrhizal infection in roots of cowpea plants and plant height after 90 days of growth in fumigated soil with residual P.

Hayman (1975) showed that nitrogen and phosphorus fertilizers have a negative effect on spore population and percent infection of VA mycorrhiza. There are many reports of similar inverse correlations between mycorrhizal development and nitrogen fertilization (Slankis, 1965; Lanowski, 1966; Mosse and Jones, 1968; Hayman, 1970). The reason for these interactions are not well known. Davey and Danielson (1968) suggest that high levels of nitrogen depress mycorrhizal development as a consequence of the host utilizing nearly all its carbohydrates. Maximum host benefit and infection can occur only when conditions are least favorable for plant growth. In high fertility situations little or no benefit may be expected from mycorrhiza. In 1975 Cooper wrote ". . . there appears to be some critical soil available P level for each species above which it grows well without mycorrhiza and below which it depends increasingly on the symbiosis." Such a critical soil P level is not known, but it may be assumed that mycorrhizal dependence diminishes when the soil P becomes adequate for a particular plant. The finding that added phosphate depresses mycorrhizal infection leads to the question of how phosphorus supply regulates mycorrhizal development. Sanders (1975) proposed two possible hypotheses: 1) "The external phase of the fungus could be adversely affected by high concentrations of phosphate in the soil solution and 2) the phosphorus supply to the host roots influences

either directly or indirectly the susceptibility of the host to infection by the mycorrhizal fungus." Since a choice between these two hypotheses could not be made by supplying P to the plants through the soil, he investigated the effect of foliar application of phosphate on mycorrhizal onion roots and showed that high P concentration in the plant brought about by foliar spray inhibited infection. The concentration of P in the plant rather than P in the soil thus seems to play an important role in mycorrhizal establishment. Menge et al.(1978) also showed that the number of chlamydospores, vesicles, arbuscules and hyphae on roots of sudangrass were not influenced by high soil P, but were inversely related to high P concentrations in the roots.

Pure cultures of VA mycorrhiza have not been used in experiments to determine the effect of mycorrhiza on plant growth because the organism cannot be cultured artificially. This has raised the question; could the growth increases reported by most workers have been brought about by contaminating microorganism associated with the inoculum rather than by mycorrhiza? For example, Gerretsen (1948) used a pure culture of bacteria isolated from the rhizosphere of oats, mustard and sunflower plants to infect sterile soil in order to verify the extent to which they could influence phosphate uptake by the plant. He showed that the amount of phosphate assimilated by the

plant as well as the weight of these plants was markedly increased by infection with the bacteria and concluded that the microorganisms had definitely made available a certain amount of phosphorus that was otherwise inaccessible.

To circumvent this problem, some workers have added washings of the mycorrhizal inoculum to the control treatments to ensure that the control contains all organisms in the inoculum except the VA mycorrhizal fungus (Gerdemann, 1964; Abbott and Robson, 1977). This process was not carried out in the present experiment. Nonetheless, it may be concluded that increase in growth and yield was caused by mycorrhiza rather than by other contaminating organisms because at the time of final harvest, plant growth was well correlated with percent mycorrhizal infection of plant roots. The biggest plants were also the ones with the highest amount of infection (Figure 12 and 13).

Effects of Nodulation

Cowpea was chosen for this experiment because it is widely grown in these soils in the tropics and also because it is quite responsive to mycorrhizal inoculations. Islam et al. (1980) reported that inoculation of cowpea plants with VA mycorrhiza caused very rapid infection of the roots. Moreover, being a legume, it offers an opportunity to evaluate the relationship between legume, Rhizobium and mycorrhiza.

All plants were inoculated with Rhizobium; however, nodulation was consistently higher in the treatments inoculated with VA mycorrhiza (Tables 11 and 12). Applied and residual soil phosphate particularly at the high levels, further improved nodulation of the inoculated plants. Roots of all plants in the non-fumigated soil had medium to high rates of nodulation, whether inoculated with mycorrhiza or not. Differences were small, however.

Understanding the tripartite symbiosis of legume-rhizobium-mycorrhiza is of particular importance. The root systems of legumes can be infected by VA mycorrhiza and by nitrogen fixing bacteria; both of them are beneficial to the plant. Legumes are capable of obtaining nitrogen through symbiotic association with rhizobia and it is common practice for farmers to grow leguminous plants on poor soils to increase fertility and save on chemical fertilizer costs. On the other hand, VA mycorrhiza has been shown to improve plant P uptake. It is therefore important to understand the path-ways and factors which may affect the uptake of P by legumes inoculated with VA mycorrhiza.

It is known that nodulation and nitrogen fixation have high phosphate requirements (Bergerson, 1971). Munns and Mosse (1980) have pointed out that the phosphate requirement of legumes may be larger than that for other plants because P is required not only for plant growth, but also for nodulation and nitrogen fixation. Inoculation

Table 11: Nodulation rating of cowpea plants inoculated with Glomus mosseae at 5 levels of applied phosphate in fumigated and non-fumigated soils (means of 3 replicates).

| Fumigation | Inoculation | Nodulation rating* Applied phosphate g/g | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 12 | 24 | 48 | 96 |
| - | - | 5.0 | 6.3 | 7.0 | 7.8 | 8.0 |
| | + | 5.0 | 6.5 | 7.0 | 8.0 | 8.0 |
| + | - | 2.0 | 2.0 | 2.5 | 5.0 | 5.5 |
| | + | 3.0 | 4.0 | 5.5 | 5.5 | 6.0 |

*See text for rating method.

Table 12: Nodulation rating of cowpea plants inoculated with Glomus mosseae at 5 levels of residual phosphate in fumigated and non-fumigated soils (means of 3 replicates).

| Fumigation | Inoculation | Nodulation rating* Residual Phosphate ( g/g) | | | | |
|---|---|---|---|---|---|---|
| | | 14 | 15 | 20 | 22 | 26 |
| - | - | 5.0 | 5.0 | 5.3 | 6.3 | 6.0 |
| | + | 5.3 | 5.3 | 5.5 | 6.3 | 6.5 |
| + | - | 2.0 | 2.0 | 2.5 | 4.0 | 5.0 |
| | + | 3.0 | 3.5 | 5.0 | 6.0 | 6.8 |

*See text for rating method.

of legumes with VA mycorrhizal fungi and Rhizobium has been reported to have synergistic beneficial effects on nodulation, nitrogen fixation and plant growth (Daft and El Giahmi, 1976; Abbott and Robson, 1977). Bagyaraj et al. (1979a) studied the effect of VA mycorrhiza on nitrogen fixation and plant growth. They compared the effect of 4 inoculation treatments on soybeans, viz 1) uninoculated controls 2) inoculation with Rhizobium japonicum, 3) inoculation with the mycorrhizal fungus Glomus fasciculatus, and 4) dual inoculation with Glomus and Rhizobium. After 60 days, nodule mass, nodule nitrogen and total shoot nitrogen content from the double inoculation treatment were twice that from single inoculation with either Glomus or Rhizobium. Their results thus suggested that VA mycorrhiza can greatly stimulate nodulation and nitrogen fixation in legumes inoculated with Rhizobium. Waidyanatha and his colleagues (1979) also reported that VA mycorrhiza stimulated nodulation and nodule activity more than plant growth in Pueraria and Stylosanthes. Mycorrhizal inoculation increased plant growth by only 17%, but increased nodule weight and nitrogenase activity by 600% and 200%, respectively. These results led them to conclude that enhancement of nitrogen fixation may be the most important effect of mycorrhiza on legumes.

The interaction between Rhizobium, nodulation and VA mycorrhizal infection is not clearly understood.

Carling et al. (1979a) reported that the first infection by mycorrhiza in soybean appeared 10-12 days after planting which was also the approximate time that Rhizobium root nodules were observed. In another investigation, the same workers (1979b) attempted to evaluate the interaction between the mycorrhizal fungus and nitrogen fixing bacteria. Nodulating and non-nodualting soybeans were treated with a combination of Glomus fasciculatus, Rhizobium and phosphate fertilizer. Dually infected, nodulating plants had higher total dry weight and nodule dry weight as well as higher levels of nitrogenase activity and nitrate reductase than non-infected plants. When phosphorus was substituted for mycorrhiza, similar growth and enzyme activity were produced. This led them to conclude that the effects resulted not from direct interaction between VA mycorrhiza and the nitrogen fixing bacteria, but rather from improved nutritional environment of the plant with each microorganism complementing the stimulatory effect of the other on the growth of the host. Crush (1974) suggested that apart from the effect on the host's phosphorus supply, VA mycorrhiza may influence the legume-rhizobium symbiosis by altering the rhizosphere environment for Rhizobia. Borea and his colleagues in 1972 found that mycorrhiza can enhance the accumulation and persistence of Azotobacter paspali in the rhizosphere of Paspalum notatum.

Effects on P Uptake

The effect of mycorrhizal inoculation on P uptake depended on whether or not the plants were growing in sterilized or unsterilized soil. Results of leaf tissue analysis in sterilized and unsterilized soil are shown in Tables 13 - 16. In general, as the soil P level increased the concentration of P in the leaves increased although there were numerous inconsistencies. Also the inoculated plants in the sterilized soil had higher concentrations of P than similarly treated plants in the unsterilized soil at all levels of applied P (Table 17). This is probably due to competition from native mycorrhiza in the unsterilized soil. Differences in P uptake between inoculated and uninoculated plants in unsterilized soil were small. Concentration of P in leaves of non-inoculated plants in unsterilized soil remained at approximately 0.13% and 0.11% for soil with newly applied P and soil with residual P, respectively,regardless of the P level (Tables 14 and 16). Non-inoculated plants in the sterilized soil lost all their leaves except at the highest P level. Consequently, no data are available for nutrient concentration in these treatments. The inoculated plants at the lowest P level were also found to take up more P than the non-inoculated plants at the highest applied P levels in both sterilized and nonsterilized soils. This result is consistent with the result obtained for plant growth

Table 13: Nutrient concentration in leaves of cowpea plants at 50% flowering which were inoculated with *Glomus mosseae* and grown in fumigated soil at 5 levels of applied P (means of 3 replicates)

| Applied P Level µg/g | Inoculation | N | P | K | Ca | Mg | S | Si | Na | Cl | Al | Mn | Fe | Cu | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Percent | | | | | | | | | ppm | | | | |
| 0 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | + | 4.31 | 0.19 | 2.83 | 1.68 | 0.39 | 0.18 | 0.14 | 0.08 | 0.37 | 4552 | 875 | 109 | 9 | 67 |
| 12 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | + | 4.28 | 0.20 | 2.91 | 1.66 | 0.39 | 0.18 | 0.14 | 0.08 | 0.41 | 4212 | 920 | 130 | 7 | 66 |
| 24 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | + | 4.46 | 0.18 | 2.59 | 1.61 | 0.38 | 0.19 | 0.22 | 0.09 | 0.41 | 4695 | 929 | 152 | 4 | 59 |
| 48 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | + | 5.18 | 0.22 | 2.64 | 1.83 | 0.38 | 0.20 | 0.11 | 0.09 | 0.40 | 5023 | 1212 | 109 | 2 | 52 |
| 96 | - | 4.50 | 0.15 | 2.82 | 1.59 | 0.57 | 0.19 | 0.16 | 0.09 | 0.36 | 3234 | 1768 | 108 | 5 | 49 |
| | + | 4.91 | 0.22 | 2.83 | 1.83 | 0.29 | 0.21 | 0.15 | 0.09 | 0.45 | 4568 | 730 | 117 | 4 | 52 |

Table 14: Nutrient concentration in leaves of cowpea plants at 50% flowering which were inoculated with Glomus mosseae and grown in non-fumigated soil at 5 levels of applied P (means of 3 replicates)

| Applied P Level µg/g | Inoculation | N | P | K | Ca | Mg | S | Si | Na | Cl | Al | Mn | Fe | Cu | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Percent | | | | | | | | | ppm | | | | |
| 0 | - | 2.69 | 0.13 | 2.25 | 1.69 | 0.28 | 0.16 | 0.15 | 0.08 | 0.64 | 1509 | 312 | 100 | 6 | 48 |
| | + | 3.26 | 0.15 | 2.14 | 1.62 | 0.34 | 0.16 | 0.13 | 0.08 | 0.52 | 2155 | 492 | 100 | 6 | 63 |
| 12 | - | 2.99 | 0.13 | 2.18 | 2.23 | 0.30 | 0.17 | 0.18 | 0.08 | 0.67 | 770 | 405 | 95 | 5 | 54 |
| | + | 3.18 | 0.15 | 2.37 | 1.61 | 0.30 | 0.15 | 0.17 | 0.09 | 0.54 | 2422 | 635 | 111 | 6 | 55 |
| 24 | - | 2.50 | 0.13 | 1.26 | 0.92 | 0.21 | 0.12 | 0.09 | 0.08 | 0.53 | 553 | 313 | 97 | 6 | 88 |
| | + | 3.38 | 0.15 | 2.19 | 1.46 | 0.28 | 0.16 | 0.16 | 0.09 | 0.45 | 4004 | 625 | 125 | 13 | 41 |
| 48 | - | 2.46 | 0.12 | 2.27 | 1.99 | 0.17 | 0.15 | 0.14 | 0.08 | 0.60 | 482 | 324 | 93 | 6 | 40 |
| | + | 3.32 | 0.15 | 1.91 | 1.49 | 0.23 | 0.14 | 0.09 | 0.08 | 0.44 | 2250 | 640 | 101 | 4 | 44 |
| 96 | - | 3.25 | 0.13 | 2.49 | 2.06 | 0.34 | 0.17 | 0.16 | 0.09 | 0.58 | 1734 | 931 | 108 | 5 | 44 |
| | + | 4.11 | 0.19 | 2.41 | 1.89 | 0.28 | 0.19 | 0.14 | 0.08 | 0.51 | 2743 | 541 | 106 | 5 | 50 |

Table 15: Nutrient concentration in leaves of cowpea plants at 50% flowering which were inoculated with *Glomus mosseae* and grown in fumigated soil at 5 levels of residual P (means of 3 replicates).

| Residual P Level µg/g | Inoculation | N | P | K | Ca | Mg | S | Si | Na | Cl | Al | Mu | Fe | Cu | Zu |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Percent | | | | | | | | | ppm | | | | |
| 14 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | + | 3.90 | 0.19 | 2.65 | 1.78 | 0.42 | 0.19 | 0.23 | 0.09 | 0.38 | 4075 | 275 | 103 | 16 | 71 |
| 15 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | + | 3.31 | 0.14 | 2.23 | 1.40 | 0.39 | 0.15 | 0.16 | 0.08 | 0.36 | 4144 | 496 | 104 | 4 | 63 |
| 20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | + | 3.90 | 0.17 | 3.09 | 1.86 | 0.34 | 0.19 | 0.29 | 0.08 | 0.48 | 4278 | 468 | 111 | 8 | 74 |
| 22 | - | 2.73 | 0.11 | 1.87 | 1.19 | 0.32 | 0.11 | 0.15 | 0.08 | 0.47 | 4554 | 943 | 94 | 1 | 60 |
| | + | 4.08 | 0.19 | 2.26 | 1.92 | 3.38 | 0.18 | 0.21 | 0.09 | 0.44 | 4981 | 354 | 113 | 6 | 75 |
| 26 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | + | 4.00 | 0.17 | 2.61 | 1.61 | 0.34 | 0.17 | 0.14 | 0.08 | 0.48 | 4147 | 475 | 101 | 5 | 63 |

Table 16: Nutrient concentration in leaves of cowpea plants at 50% flowering which were inoculated with Glomus mosseae and grown in non-fumigated soil at 5 levels of residual P (means of 3 replicates).

| Residual P level µg/g | Inoculation | N | P | K | Ca | Mg | S | Si | Na | Cl | Al | Mu | Fe | Cu | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Percent | | | | | | | | | ppm | | | | |
| 14 | - | 2.71 | 0.11 | 2.14 | 1.31 | 0.32 | 0.14 | 0.14 | 0.08 | 0.50 | 767 | 304 | 105 | 7 | 50 |
| | + | 3.12 | 0.16 | 2.21 | 1.71 | 0.39 | 0.17 | 0.19 | 0.09 | 0.50 | 1603 | 297 | 99 | 10 | 63 |
| 15 | - | 2.21 | 0.11 | 1.59 | 1.06 | 0.23 | 0.12 | 0.09 | 0.08 | 0.55 | 1365 | 205 | 94 | 6 | 44 |
| | + | 2.83 | 0.13 | 2.08 | 1.51 | 0.34 | 0.16 | 0.15 | 0.08 | 0.61 | 1831 | 348 | 96 | 5 | 56 |
| 20 | - | 2.45 | 0.12 | 1.90 | 2.03 | 0.32 | 0.15 | 0.23 | 0.09 | 0.65 | 1141 | 427 | 106 | 4 | 57 |
| | + | 3.22 | 0.15 | 2.34 | 1.77 | 0.37 | 0.18 | 0.19 | 0.08 | 0.67 | 1634 | 324 | 109 | 6 | 62 |
| 22 | - | 2.41 | 0.11 | 1.79 | 1.35 | 0.30 | 0.13 | 0.12 | 0.08 | 0.51 | 1702 | 467 | 91 | 3 | 50 |
| | + | 2.97 | 0.15 | 2.27 | 1.96 | 0.35 | 0.16 | 0.20 | 0.08 | 0.55 | 2952 | 370 | 110 | 5 | 68 |
| 26 | - | 2.54 | 0.13 | 1.94 | 1.80 | 0.30 | 0.16 | 0.13 | 0.08 | 0.75 | 963 | 331 | 95 | 5 | 49 |
| | + | 3.18 | 0.15 | 2.20 | 1.69 | 0.33 | 0.16 | 0.16 | 0.08 | 0.49 | 2441 | 372 | 99 | 4 | 54 |

Table 17: Effect of applied P on percent P concentration in leaves of cowpea plants inoculated with Glomus mosseae and grown in fumigated and non-fumigated soils (means of 3 replicates).

| Fumigation | Inoculation | Applied P Level (µg/g) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 12 | 24 | 48 | 96 |
| - | + | 0.15 | 0.15 | 0.15 | 0.15 | 0.19 |
| + | + | 0.19 | 0.20 | 0.18 | 0.22 | 0.22 |

at final harvest (Tables 7 and 8) where inoculated plants at the lowest P level grew better than the non-inoculated plants at the highest levels of P, an indication that plants benefit more from mycorrhizal infections when soil P is low.

The advantage conferred by inoculation with VA mycorrhiza in unsterilized soil was greater in the soil with residual P than in the soil with newly applied P. This was manifested at the lowest P level where plant P concentration in inoculated plants grown in soil with residual P was 45.5% greater than that of non-inoculated plants (Table 16) compared to 15.4% in the soil with newly applied P (Table 14). The relative advantage of mycorrhizal inoculations in unsterilized soil will depend on the level of native mycorrhizal population as well as on their efficiency. The advantage will be greatest if the indigenous population is low or inefficient. This raises the question of the inoculum level in natural soil at which inoculation would benefit plant growth. The answer to this question is unclear.

The finding that mycorrhizal plants at the lowest P level in unsterilized soil took up as much P as those at higher P levels (Table 17) confirms the theory that the mycorrhizal effect is greatest in soils containing little available phosphate (Mosse, 1973a). At high P levels mycorrhiza may actually have a deleterious effect on plant

growth. Baylis (1967) noted a depression of growth due to mycorrhiza in Coprosma in high P situations. Also depression of growth of inoculated onions led Furlan and Fortin (1973) to conclude that the mycorrhizal inoculum was parasitic. Crush (1976) found that mycorrhiza reduced growth of Trifolium hybridum and Medicago sativa by 3 - 16% and concluded that the endophyte-host relationship in the symbiosis can change from mutualism to parasitism as phosphorus availability increases. Ross (1971) found that mycorrhizal soybeans grown under field conditions weighed more and yielded more seed with an application of 44 Kg P/ha than with 176 Kg P/ha. Mycorrhizal maize and wheat plants grown without added phosphate grew better than similarly treated plants with added phosphate (Khan, 1972, 1973). Mosse (1973b) attributed this effect to accumulation of supra-optimal concentrations of P in mycorrhizal plants leading to P toxicity and hence depressed growth while Cooper (1975) interpreted the growth depression as a transitory phase in the establishment of the endophyte.

Another attribute of mycorrhiza is that mycorrhizal roots may act as storage organs of applied phosphate absorbed before it becomes immobilized in the soil (Mosse, 1977b). Evidence for this is provided by results of the work of Ling-lee et al. (1975) who showed that phosphate is stored and transported by the fungal component in the form of polyphosphate granules.

In addition to enabling plants to absorb P from a larger volume of soil, mycorrhiza may also ensure a more complete exhaustion of P from the volume of soil explored by the hyphae, including the depletion zone (Mosse et al., 1973). It follows, therefore, that after a mycorrhizal crop available P reserves in the soil will be depleted since, as Mosse (1977) points out, "insoluble P will only go into solution when the concentration of the soil solution falls below the solubility product of the insoluble P." It has previously been shown (Stout and Overstreet, 1954) that phosphate in solution can be replenished several times a day following the growth of plants in soils containing 1 ppm phosphate in solution. Soil depletion of P following the growth of a mycorrhizal crop has been demonstrated by Gerdemann (1964) with soil analysis at the end of an experiment. Soil in which mycorrhizal corn plants were grown had significantly less P after harvesting the experiment than before planting it.

Uptake of Nutrients Other than Phosphorus

The soils for these experiments were fertilized with a nutrient solution containing Mg, S, Cu, Fe, Mn, Zn, B and Mo to ensure uniform growth. Therefore, measurement of mycorrhizal effects on uptake of these nutrients was not intended. Nevertheless, the data in Tables 13-16 indicate that mycorrhizal inoculations also led to increased uptake of nutrients other than P in both sterilized and unsterilized

soils, notably N, K, Ca, Mg, S, Al, Mn, Fe, Cu and Zn. Differences in concentration of these nutrients were significant at 5% level though there were inconsistencies between the soils with newly applied and residual P (Appendix Table 29). Ross and Harper (1970) found that mycorrhizal soybeans contained greater amounts of N, Ca, Cu, and Mn in their foliage than non-mycorrhizal plants. Inoculation of peach seedlings with VA mycorrhiza eliminated the appearance of Zn deficiency symptoms and mycorrhizal seedlings had 2 - 3 times more Zn in tops than did the controls (Gilmore, 1971). Gray and Gerdemann (1973) demonstrated that VA mycorrhizal plants took up sulfur more efficiently than did comparable non-mycorrhizal plants. However, results reported in the literature are not consistent.

# V. SUMMARY AND CONCLUSIONS

Two experiments were conducted in the green house in the Dept of Agronomy and Soil Science, University of Hawaii to investigate the relationship of soil inoculum level to infectivity and to evaluate the contribution of VA mycorrhiza to the growth and P uptake of cowpea in a Tropeptic Eutrustox. The method of spore count and soil dilution bioassay were used to estimate soil inoculum potential. A pot study of the contribution of VA mycorrhiza to plant growth was carried out using cowpea that was inoculated with Glomus mosseae and grown to maturity. Growth variables measured included plant height, stover weight, root weight, pod and seed yield, nodulation, root infection, and nutrient concentration in leaves sampled at 50% flowering. From the results obtained the following conclusions may be drawn.

1. On a soil volume basis 5 spores/g of soil found in this study may be considered adequate for reasonable infection of plant roots. This was confirmed in the soil dilution experiment in which infection occurred at soil dilutions as high as 1/256.
2. The soil dilution method can be used to approximate natural infectivity of the soil provided care is taken to mix the soils of the dilution series thoroughly.

3. Great difficulties are involved in objectively assessing inoculum level of the soil using a test plant because of unexpected variations in growth conditions of the test plant. As far as possible conditions in the growth environment should be made uniform to ensure uniform growth of the test plant.

4. Clear benefits to mycorrhizal infections have been demonstrated. The results extend and confirm the conclusions of many investigators that mycorrhizae contribute to the growth and P uptake of plants in soils with low P. Plant height, pod and seed yield, nodulation and P concentration in leaves were significantly increased by mycorrhizal inoculation. Uptake of nutrients other than P were also enhanced by mycorrhizal inoculation notably N, K, Mg, S, Si, Al, Mn, Fe and Zn. The greatest contribution of mycorrhiza seems to be related to its ability to alleviate some constraints to plant acquisition of nutrients from the soil. In this experiment mycorrhizal inoculation alleviated growth depressions due to methyl bromide fumigation. The data also suggest that mycorrhiza may be involved in overcoming mineral toxicities in plants particularly Al and Mn.

5. The mycorrhizal effect was greatest in fumigated soils. In non-fumigated soil, the effect was less

pronounced due to presence of indigenous mycorrhiza.

# A P P E N D I X

Table 18: ORIGINAL MEASUREMENTS OF GROWTH VARIABLES AND NUTRIENT CONCENTRATIONS OF COWPEA PLANTS GROWN IN FIVE DILUTIONS OF NON-STERILE TO STERILE SOIL

| | | | | (cm) | (g/pot) | (g/pot) | | percent | | | | | | | | ppm | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OBS | TRT | REP | DIL | HT | STW | RTW | INF | N | P | K | CA | MG | S | SI | NA | MN | FE | CU | ZN |
| 1 | 8 | 1 | 1 | 12.5 | 2.05 | 1.70 | 35 | 1.90 | 0.12 | 2.43 | 1.72 | 0.34 | 0.18 | 0.48 | 0.08 | 303 | 281 | 5 | 43 |
| 2 | 8 | 1 | 2 | 15.0 | 2.65 | 1.90 | 30 | 2.46 | 0.11 | 2.43 | 1.44 | 0.31 | 0.13 | 0.37 | 0.08 | 344 | 170 | 4 | 37 |
| 3 | 8 | 1 | 3 | 14.0 | 1.75 | 1.45 | 20 | 2.72 | 0.11 | 2.26 | 1.46 | 0.32 | 0.19 | 0.35 | 0.08 | 403 | 175 | 4 | 33 |
| 4 | 8 | 1 | 4 | 12.8 | 2.20 | 2.10 | 10 | 2.67 | 0.10 | 2.02 | 1.26 | 0.27 | 0.21 | 0.29 | 0.08 | 323 | 174 | 7 | 28 |
| 5 | 8 | 1 | 5 | 13.6 | 1.75 | 1.55 | 5 | 2.49 | 0.11 | 2.22 | 1.30 | 0.28 | 0.19 | 0.36 | 0.08 | 384 | 166 | 3 | 37 |
| 6 | 8 | 2 | 1 | 15.0 | 2.05 | 2.10 | 30 | 1.72 | 0.11 | 2.02 | 1.31 | 0.30 | 0.14 | 0.35 | 0.07 | 233 | 154 | 3 | 30 |
| 7 | 8 | 2 | 2 | 13.9 | 1.95 | 1.85 | 33 | 2.06 | 0.12 | 2.75 | 1.46 | 0.33 | 0.15 | 0.37 | 0.07 | 272 | 323 | 3 | 33 |
| 8 | 8 | 2 | 3 | 19.4 | 2.85 | 2.35 | 15 | 2.39 | 0.10 | 2.31 | 1.41 | 0.26 | 0.18 | 0.43 | 0.09 | 368 | 167 | 6 | 39 |
| 9 | 8 | 2 | 4 | 13.5 | 2.65 | 2.10 | 10 | 2.55 | 0.11 | 2.22 | 1.22 | 0.28 | 0.19 | 0.34 | 0.07 | 356 | 167 | 5 | 31 |
| 10 | 8 | 2 | 5 | 16.9 | 2.90 | 2.40 | 5 | 2.49 | 0.11 | 2.21 | 1.16 | 0.25 | 0.18 | 0.38 | 0.08 | 363 | 160 | 6 | 36 |
| 11 | 8 | 3 | 1 | 13.7 | 2.35 | 2.00 | 35 | . | . | . | . | . | . | . | . | . | . | . | . |
| 12 | 8 | 3 | 2 | 13.5 | 1.10 | 1.90 | 33 | . | . | . | . | . | . | . | . | . | . | . | . |
| 13 | 8 | 3 | 3 | 18.5 | 3.70 | 2.70 | 20 | . | . | . | . | . | . | . | . | . | . | . | . |
| 14 | 8 | 3 | 4 | 13.7 | 1.45 | 1.45 | 10 | . | . | . | . | . | . | . | . | . | . | . | . |
| 15 | 8 | 3 | 5 | 10.5 | 2.65 | 2.50 | 5 | . | . | . | . | . | . | . | . | . | . | . | . |
| 16 | 8 | 4 | 1 | 10.3 | 1.55 | 2.00 | 35 | . | . | . | . | . | . | . | . | . | . | . | . |
| 17 | 8 | 4 | 2 | 10.6 | 2.65 | 2.45 | 30 | . | . | . | . | . | . | . | . | . | . | . | . |
| 18 | 8 | 4 | 3 | 11.7 | 2.15 | 2.15 | 20 | . | . | . | . | . | . | . | . | . | . | . | . |
| 19 | 8 | 4 | 4 | 17.6 | 3.60 | 2.95 | 10 | . | . | . | . | . | . | . | . | . | . | . | . |
| 20 | 8 | 4 | 5 | 15.2 | 2.95 | 2.35 | 5 | . | . | . | . | . | . | . | . | . | . | . | . |
| 21 | 18 | 1 | 1 | 18.9 | 3.30 | 2.25 | 30 | 1.73 | 0.10 | 1.83 | 1.48 | 0.26 | 0.15 | 0.37 | 0.08 | 270 | 148 | 6 | 35 |
| 22 | 18 | 1 | 2 | 15.9 | 2.80 | 2.00 | 20 | 2.20 | 0.10 | 2.24 | 1.64 | 0.27 | 0.14 | 0.32 | 0.09 | 329 | 159 | 6 | 39 |
| 23 | 18 | 1 | 3 | 15.0 | 1.00 | 1.90 | 10 | 2.71 | 0.11 | 2.39 | 1.31 | 0.25 | 0.22 | 0.35 | 0.08 | 270 | 148 | 2 | 35 |
| 24 | 18 | 1 | 4 | 16.3 | 2.25 | 2.15 | 5 | 2.90 | 0.11 | 2.64 | 1.61 | 0.26 | 0.26 | 0.46 | 0.08 | 388 | 184 | 0 | 44 |
| 25 | 18 | 1 | 5 | 14.0 | 2.20 | 2.10 | 3 | 2.31 | 0.10 | 2.19 | 1.28 | 0.29 | 0.22 | 0.32 | 0.08 | 381 | 155 | 5 | 41 |
| 26 | 18 | 2 | 1 | 15.3 | 2.50 | 2.30 | 30 | 1.32 | 0.11 | 2.20 | 1.38 | 0.29 | 0.13 | 0.44 | 0.08 | 256 | 156 | 2 | 36 |
| 27 | 18 | 2 | 2 | 16.3 | 2.80 | 2.45 | 20 | 1.83 | 0.11 | 2.16 | 1.25 | 0.33 | 0.13 | 0.32 | 0.07 | 230 | 156 | 3 | 26 |
| 28 | 18 | 2 | 3 | 16.0 | 2.70 | 2.50 | 15 | 1.73 | 0.10 | 2.13 | 1.23 | 0.27 | 0.18 | 0.30 | 0.08 | 350 | 151 | 4 | 29 |
| 29 | 18 | 2 | 4 | 11.0 | 1.45 | 2.35 | 5 | 2.36 | 0.10 | 1.80 | 1.07 | 0.32 | 0.16 | 0.26 | 0.07 | 270 | 165 | 1 | 22 |
| 30 | 18 | 2 | 5 | 15.6 | 2.65 | 2.10 | 2 | 2.62 | 0.11 | 2.44 | 1.33 | 0.28 | 0.24 | 0.42 | 0.08 | 350 | 163 | 3 | 40 |
| 31 | 18 | 3 | 1 | 15.8 | 2.20 | 2.65 | 30 | . | . | . | . | . | . | . | . | . | . | . | . |
| 32 | 18 | 3 | 2 | 18.5 | 2.60 | 2.40 | 20 | . | . | . | . | . | . | . | . | . | . | . | . |
| 33 | 18 | 3 | 3 | 20.5 | 4.20 | 2.85 | 10 | . | . | . | . | . | . | . | . | . | . | . | . |
| 34 | 18 | 3 | 4 | 14.6 | 2.40 | 2.25 | 5 | . | . | . | . | . | . | . | . | . | . | . | . |
| 35 | 18 | 3 | 5 | 14.6 | 2.40 | 2.20 | 3 | . | . | . | . | . | . | . | . | . | . | . | . |
| 36 | 18 | 4 | 1 | 17.0 | 3.10 | 2.55 | 35 | . | . | . | . | . | . | . | . | . | . | . | . |
| 37 | 18 | 4 | 2 | 15.5 | 3.05 | 2.65 | 20 | . | . | . | . | . | . | . | . | . | . | . | . |
| 38 | 18 | 4 | 3 | 20.8 | 3.80 | 2.85 | 10 | . | . | . | . | . | . | . | . | . | . | . | . |
| 39 | 18 | 4 | 4 | 16.1 | 3.00 | 2.60 | 5 | . | . | . | . | . | . | . | . | . | . | . | . |
| 40 | 18 | 4 | 5 | 12.1 | 2.05 | 1.95 | 3 | . | . | . | . | . | . | . | . | . | . | . | . |
| 41 | 23 | 1 | 1 | 11.8 | 1.65 | 1.75 | 25 | 2.39 | 0.11 | 2.24 | 2.01 | 0.34 | 0.14 | 0.41 | 0.09 | 297 | 151 | 4 | 35 |
| 42 | 23 | 1 | 2 | 16.1 | 2.70 | 2.05 | 10 | 2.37 | 0.11 | 2.61 | 1.21 | 0.37 | 0.23 | 0.32 | 0.09 | 332 | 150 | 6 | 47 |
| 43 | 23 | 1 | 3 | 12.0 | 1.70 | 1.60 | 5 | 2.83 | 0.13 | 2.34 | 1.45 | 0.33 | 0.15 | 0.40 | 0.08 | 315 | 176 | 3 | 44 |
| 44 | 23 | 1 | 4 | 12.7 | 2.30 | 2.55 | 5 | 2.66 | 0.11 | 2.62 | 1.24 | 0.34 | 0.22 | 0.36 | 0.08 | 376 | 229 | 3 | 44 |
| 45 | 23 | 1 | 5 | 18.8 | 3.40 | 2.40 | 3 | 2.17 | 0.10 | 2.04 | 1.29 | 0.27 | 0.20 | 0.40 | 0.07 | 365 | 172 | 2 | 32 |
| 46 | 23 | 2 | 1 | 16.8 | 2.80 | 2.20 | 20 | 2.73 | 0.10 | 2.44 | 1.74 | 0.35 | 0.16 | 0.31 | 0.08 | 270 | 162 | 3 | 36 |
| 47 | 23 | 2 | 2 | 17.2 | 2.55 | 2.55 | 15 | 1.78 | 0.10 | 2.04 | 2.04 | 0.26 | 0.12 | 0.50 | 0.06 | 199 | 152 | 4 | 25 |
| 48 | 23 | 2 | 3 | 13.2 | 1.95 | 2.20 | 5 | 2.67 | 0.11 | 2.36 | 1.55 | 0.35 | 0.19 | 0.47 | 0.08 | 313 | 168 | 3 | 40 |
| 49 | 23 | 2 | 4 | 14.8 | 2.00 | 1.95 | 5 | 2.05 | 0.10 | 2.23 | 1.50 | 0.23 | 0.19 | 0.39 | 0.07 | 303 | 172 | 1 | 31 |
| 50 | 23 | 2 | 5 | 14.8 | 2.60 | 2.10 | 3 | 2.24 | 0.10 | 2.35 | 1.24 | 0.28 | 0.18 | 0.35 | 0.08 | 396 | 162 | 5 | 40 |
| 51 | 23 | 3 | 1 | 0.6 | 2.10 | 25.00 | 23 | . | . | . | . | . | . | . | . | . | . | . | . |
| 52 | 23 | 3 | 3 | 13.6 | 2.55 | 2.10 | 10 | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 23 | 3 | 4 | 16.2 | 2.90 | 2.45 | 5 | . | . | . | . | . | . | . | . | . | . | . | . |
| 54 | 23 | 3 | 5 | 14.9 | 2.65 | 2.30 | 3 | . | . | . | . | . | . | . | . | . | . | . | . |
| 55 | 23 | 4 | 1 | 15.0 | 2.35 | 2.35 | 25 | . | . | . | . | . | . | . | . | . | . | . | . |
| 56 | 23 | 4 | 2 | 16.5 | 3.55 | 3.00 | 15 | . | . | . | . | . | . | . | . | . | . | . | . |
| 57 | 23 | 4 | 3 | 14.0 | 2.05 | 1.95 | 10 | . | . | . | . | . | . | . | . | . | . | . | . |
| 58 | 23 | 4 | 4 | 17.0 | 3.40 | 3.40 | 3 | . | . | . | . | . | . | . | . | . | . | . | . |
| 59 | 23 | 4 | 5 | 14.8 | 2.70 | 2.40 | 3 | . | . | . | . | . | . | . | . | . | . | . | . |

DIL 1 = 1/10, DIL 2 = 1/4, DIL 3 = 1/16, DIL 4 = 1/64, DIL 5 = 1/256, TRT = ug P/g.

Table 19: ORIGINAL MEASUREMENTS OF GROWTH VARIABLES OF MYCORRHIZAL AND NON-MYCORRHIZAL COWPEA PLANTS GROWN IN STERILIZED AND UNSTERILIZED SOILS AT FIVE LEVELS OF APPLIED PHOSPHATE

| | | | | ----------cm---------- | | | (g/pot) | (g/pot) | no. | % | (no/plant) | (g/plant) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| REP | FUM | INOC | TRT | HT1 | HT2 | HT3 | RTW | STW | NOD | INF | PODN | SEEDW |
| 1 | 0 | 0 | 0 | 30.5 | . | . | 3.40 | 8.90 | 5.0 | 60 | 2 | 2.80 |
| 1 | 0 | 0 | 12 | 28.0 | . | . | 3.75 | 14.30 | 6.5 | 50 | 3 | 3.00 |
| 1 | 0 | 0 | 24 | 31.8 | . | . | 3.70 | 9.20 | 7.0 | 40 | 4 | 2.80 |
| 1 | 0 | 0 | 48 | 32.2 | . | . | 3.65 | 12.70 | 7.5 | 40 | 5 | 4.55 |
| 1 | 0 | 0 | 96 | 32.4 | . | . | 4.20 | 14.20 | 8.0 | 30 | 5 | 4.55 |
| 1 | 0 | 1 | 0 | 30.8 | . | . | 4.55 | 9.40 | 5.0 | 65 | 3 | 3.15 |
| 1 | 0 | 1 | 12 | 29.1 | . | . | 4.75 | 10.40 | 6.5 | 65 | 3 | 4.65 |
| 1 | 0 | 1 | 24 | 29.8 | . | . | 3.90 | 8.80 | 7.3 | 55 | 4 | 4.00 |
| 1 | 0 | 1 | 48 | 33.3 | . | . | 4.40 | 13.10 | 8.0 | 50 | 3 | 5.80 |
| 1 | 0 | 1 | 96 | 33.0 | . | . | 4.15 | 14.60 | 8.0 | 40 | 4 | 5.20 |
| 1 | 1 | 0 | 0 | 9.2 | 14.8 | 14.0 | 0.88 | 1.75 | 2.0 | 0 | 0 | 0.00 |
| 1 | 1 | 0 | 12 | 10.2 | 11.0 | 15.0 | 1.60 | 1.45 | 2.3 | 0 | 0 | 0.00 |
| 1 | 1 | 0 | 24 | 17.5 | 20.6 | 14.9 | 2.20 | 2.10 | 2.5 | 0 | 0 | 0.00 |
| 1 | 1 | 0 | 48 | 18.3 | 20.7 | 10.3 | 2.60 | 2.05 | 5.0 | 0 | 0 | 0.00 |
| 1 | 1 | 0 | 96 | 21.3 | 28.0 | 24.0 | 3.00 | 1.55 | 5.5 | 0 | 2 | 1.90 |
| 1 | 1 | 1 | 0 | 11.6 | 17.5 | 33.0 | 1.00 | 1.90 | 3.0 | 70 | 1 | 2.60 |
| 1 | 1 | 1 | 12 | 11.0 | 17.0 | 25.5 | 2.15 | 5.15 | 4.0 | 75 | 2 | 1.45 |
| 1 | 1 | 1 | 24 | 20.3 | 20.9 | 45.0 | 2.08 | 6.50 | 5.0 | 70 | 2 | 2.30 |
| 1 | 1 | 1 | 48 | 17.9 | 17.5 | 40.3 | 1.65 | 4.90 | 5.5 | 50 | 3 | 3.10 |
| 1 | 1 | 1 | 96 | 17.3 | 32.3 | 35.5 | 3.42 | 4.70 | 6.0 | 45 | 4 | 4.45 |
| 2 | 0 | 0 | 0 | 34.5 | . | . | 1.75 | 19.70 | 5.0 | 60 | 3 | 2.70 |
| 2 | 0 | 0 | 12 | 34.9 | . | . | 3.90 | 16.50 | 6.0 | 60 | 5 | 4.65 |
| 2 | 0 | 0 | 24 | 36.3 | . | . | 5.45 | 20.90 | 7.0 | 40 | 5 | 4.80 |
| 2 | 0 | 0 | 48 | 36.8 | . | . | 4.95 | 17.60 | 8.0 | 50 | 5 | 3.25 |
| 2 | 0 | 0 | 96 | 38.6 | . | . | 6.40 | 20.60 | 8.0 | 30 | 6 | 6.10 |
| 2 | 0 | 1 | 0 | 37.3 | . | . | 2.85 | 20.80 | 5.0 | 65 | 3 | 3.05 |
| 2 | 0 | 1 | 12 | 35.2 | . | . | 4.40 | 25.30 | 6.5 | 65 | 4 | 4.13 |
| 2 | 0 | 1 | 24 | 36.4 | . | . | 5.15 | 20.30 | 7.3 | 55 | 5 | 5.70 |
| 2 | 0 | 1 | 48 | 37.3 | . | . | 4.60 | 17.00 | 8.0 | 50 | 5 | 4.60 |
| 2 | 0 | 1 | 96 | 39.4 | . | . | 7.25 | 18.20 | 8.0 | 40 | 6 | 6.00 |
| 2 | 1 | 0 | 0 | 19.8 | 21.8 | 15.8 | 1.00 | 1.25 | 2.0 | 0 | 0 | 0.00 |
| 2 | 1 | 0 | 12 | 18.6 | 20.5 | 17.0 | 2.00 | 1.10 | 2.0 | 0 | 0 | 0.00 |
| 2 | 1 | 0 | 24 | 20.2 | 24.0 | 21.6 | 2.00 | 0.65 | 2.5 | 0 | 0 | 0.00 |
| 2 | 1 | 0 | 48 | 20.0 | 26.3 | 29.0 | 2.60 | 1.50 | 5.0 | 0 | 0 | 0.00 |
| 2 | 1 | 0 | 96 | 24.5 | 24.0 | 22.7 | 2.30 | 1.60 | 5.5 | 0 | 2 | 1.40 |
| 2 | 1 | 1 | 0 | 19.3 | 29.0 | 20.3 | 1.26 | 2.56 | 3.0 | 70 | 2 | 0.60 |
| 2 | 1 | 1 | 12 | 19.3 | 31.3 | 34.4 | 3.05 | 4.00 | 4.0 | 75 | 2 | 2.10 |
| 2 | 1 | 1 | 24 | 22.5 | 40.6 | 41.4 | 4.00 | 6.85 | 6.0 | 70 | 3 | 4.00 |
| 2 | 1 | 1 | 48 | 18.5 | 34.6 | 40.0 | 4.10 | 1.15 | 5.5 | 50 | 3 | 2.50 |
| 2 | 1 | 1 | 96 | 22.5 | 30.0 | 43.5 | 2.00 | 7.80 | 6.0 | 45 | 3 | 2.40 |
| 3 | 0 | 0 | 0 | 23.5 | . | . | . | . | . | . | . | . |
| 3 | 0 | 0 | 12 | 31.5 | . | . | . | . | . | . | . | . |
| 3 | 0 | 0 | 24 | 23.9 | . | . | . | . | . | . | . | . |
| 3 | 0 | 0 | 48 | 34.5 | . | . | . | . | . | . | . | . |
| 3 | 0 | 0 | 96 | 31.6 | . | . | . | . | . | . | . | . |
| 3 | 0 | 1 | 0 | 22.0 | . | . | . | . | . | . | . | . |
| 3 | 0 | 1 | 12 | 32.1 | . | . | . | . | . | . | . | . |
| 3 | 0 | 1 | 24 | 25.3 | . | . | . | . | . | . | . | . |
| 3 | 0 | 1 | 48 | 35.3 | . | . | . | . | . | . | . | . |
| 3 | 0 | 1 | 96 | 36.0 | . | . | . | . | . | . | . | . |
| 3 | 1 | 0 | 0 | 10.2 | 14.7 | . | . | 0.95 | . | 0 | 0 | 0.00 |
| 3 | 1 | 0 | 12 | 11.3 | 13.8 | . | . | 1.25 | . | 0 | 0 | 0.00 |
| 3 | 1 | 0 | 24 | 10.9 | 11.8 | . | . | 1.45 | . | 0 | 0 | 0.00 |
| 3 | 1 | 0 | 48 | 18.0 | 18.5 | . | . | 2.20 | . | 0 | 0 | 0.00 |
| 3 | 1 | 0 | 96 | 17.0 | 18.2 | . | . | 4.45 | . | 0 | 2 | 2.00 |
| 3 | 1 | 1 | 0 | 13.0 | 13.3 | . | . | 3.35 | . | 70 | 2 | 2.25 |
| 3 | 1 | 1 | 12 | 13.0 | 14.5 | . | . | 3.70 | . | 75 | 1 | 1.25 |
| 3 | 1 | 1 | 24 | 12.7 | 13.5 | . | . | 3.45 | . | 70 | 2 | 2.75 |
| 3 | 1 | 1 | 48 | 19.3 | 20.1 | . | . | 9.05 | . | 50 | 3 | 3.10 |
| 3 | 1 | 1 | 96 | 17.3 | 21.0 | . | . | 2.70 | . | 45 | 4 | 4.67 |
| 4 | 1 | 0 | 0 | 17.4 | 11.5 | . | . | . | . | 0 | 0 | 0.00 |
| 4 | 1 | 0 | 12 | 14.8 | 15.0 | . | . | . | . | 0 | 0 | 0.00 |
| 4 | 1 | 0 | 24 | 15.3 | 17.0 | . | . | . | . | 0 | 0 | 0.00 |
| 4 | 1 | 0 | 48 | 17.2 | 19.3 | . | . | . | . | 0 | 0 | 0.00 |
| 4 | 1 | 0 | 96 | 18.8 | 17.2 | . | . | . | . | 0 | 1 | 1.76 |
| 4 | 1 | 1 | 0 | 17.9 | 18.9 | . | . | . | . | 70 | 1 | 1.80 |
| 4 | 1 | 1 | 12 | 17.2 | 17.5 | . | . | . | . | 75 | 3 | 1.14 |
| 4 | 1 | 1 | 24 | 13.6 | 26.0 | . | . | . | . | 70 | 2 | 1.93 |
| 4 | 1 | 1 | 48 | 17.9 | 37.7 | . | . | . | . | 50 | 4 | 3.70 |
| 4 | 1 | 1 | 96 | 16.3 | 22.0 | . | . | . | . | 45 | 4 | 5.05 |

FUM 0 = non-fumigated, FUM 1 = fumigated, INOC 0 = non-inoculated, INOC 1 = inoculated, TRT = ug P/g,

Table 20: NUTRIENT ANALYSES OF LEAVES OF MYCORRHIZAL AND NON-MYCORRHIZAL COWPEA PLANTS SAMPLED AT 50% FLOWERING AND GROWN IN STERILIZED AND UNSTERILIZED SOILS AT FIVE LEVELS OF APPLIED PHOSPHATE

| | | | | percent | | | | | | | | | ppm | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| REP | FUM | INOC | TRT | N | P | K | CA | MG | S | SI | NA | CL | AL | MN | FE | CU | ZN |
| 1 | 0 | 0 | 0 | 2.18 | 0.11 | 1.88 | 1.27 | 0.23 | 0.13 | 0.09 | 0.08 | 0.65 | 812 | 267 | 95 | 6 | 43 |
| 1 | 0 | 0 | 12 | 2.99 | 0.13 | 2.18 | 2.23 | 0.30 | 0.17 | 0.18 | 0.08 | 0.67 | 770 | 405 | 95 | 5 | 54 |
| 1 | 0 | 0 | 24 | 2.76 | 0.14 | 0.81 | 0.51 | 0.28 | 0.15 | 0.10 | 0.08 | 0.61 | 722 | 284 | 94 | 5 | 44 |
| 1 | 0 | 0 | 48 | 2.70 | 0.13 | 2.66 | 2.27 | 0.20 | 0.17 | 0.14 | 0.09 | 0.64 | 812 | 267 | 95 | 6 | 43 |
| 1 | 0 | 0 | 96 | 2.77 | 0.14 | 1.95 | 2.04 | 0.26 | 0.18 | 0.13 | 0.08 | 0.66 | 1475 | 400 | 127 | 6 | 44 |
| 1 | 0 | 1 | 0 | 2.74 | 0.13 | 1.79 | 1.60 | 0.32 | 0.15 | 0.12 | 0.08 | 0.60 | 957 | 301 | 96 | 5 | 61 |
| 1 | 0 | 1 | 12 | 2.08 | 0.10 | 1.84 | 1.57 | 0.22 | 0.13 | 0.15 | 0.09 | 0.68 | 633 | 351 | 93 | 5 | 45 |
| 1 | 0 | 1 | 24 | 2.77 | 0.13 | 1.94 | 1.21 | 0.24 | 0.13 | 0.10 | 0.09 | 0.43 | 1063 | 294 | 97 | 8 | 45 |
| 1 | 0 | 1 | 48 | 2.80 | 0.12 | 1.61 | 1.50 | 0.17 | 0.13 | 0.10 | 0.08 | 0.55 | 1506 | 351 | 95 | 3 | 39 |
| 1 | 0 | 1 | 96 | 3.19 | 0.19 | 2.16 | 2.06 | 0.29 | 0.18 | 0.13 | 0.08 | 0.54 | 226 | 340 | 97 | 6 | 49 |
| 2 | 0 | 0 | 0 | 3.19 | 0.14 | 2.63 | 2.11 | 0.32 | 0.18 | 0.20 | 0.08 | 0.62 | 2206 | 357 | 105 | 6 | 53 |
| 2 | 0 | 0 | 12 | 2.99 | 0.13 | 2.18 | 2.23 | 0.30 | 0.17 | 0.18 | 0.08 | 0.67 | 770 | 405 | 95 | 5 | 54 |
| 2 | 0 | 0 | 24 | 2.24 | 0.11 | 1.68 | 1.33 | 0.14 | 0.11 | 0.09 | 0.08 | 0.44 | 385 | 341 | 101 | 6 | 132 |
| 2 | 0 | 0 | 48 | 2.21 | 0.11 | 1.88 | 1.72 | 0.13 | 0.12 | 0.13 | 0.08 | 0.57 | 152 | 381 | 92 | 5 | 38 |
| 2 | 0 | 0 | 96 | 2.49 | 0.11 | 2.71 | 2.56 | 0.19 | 0.15 | 0.19 | 0.09 | 0.73 | 494 | 626 | 91 | 3 | 39 |
| 2 | 0 | 1 | 0 | 2.74 | 0.13 | 1.79 | 1.60 | 0.32 | 0.15 | 0.12 | 0.08 | 0.60 | 957 | 301 | 96 | 5 | 61 |
| 2 | 0 | 1 | 12 | 2.08 | 0.10 | 1.84 | 1.57 | 0.22 | 0.13 | 0.15 | 0.09 | 0.68 | 633 | 351 | 93 | 5 | 45 |
| 2 | 0 | 1 | 24 | 1.82 | 0.10 | 1.65 | 1.43 | 0.13 | 0.11 | 0.11 | 0.08 | 0.53 | 5562 | 348 | 101 | 40 | 2 |
| 2 | 0 | 1 | 48 | 1.97 | 0.11 | 1.48 | 1.14 | 0.16 | 0.09 | 0.06 | 0.08 | 0.36 | 223 | 354 | 101 | 6 | 42 |
| 2 | 0 | 1 | 96 | 3.42 | 0.13 | 1.82 | 1.86 | 0.24 | 0.17 | 0.13 | 0.08 | 0.59 | 1611 | 365 | 92 | 4 | 40 |
| 1 | 1 | 0 | 96 | 4.50 | 0.15 | 2.82 | 1.59 | 0.57 | 0.19 | 0.16 | 0.09 | 0.36 | 3234 | 1768 | 108 | 5 | 49 |
| 1 | 1 | 1 | 0 | 4.31 | 0.19 | 2.83 | 1.68 | 0.39 | 0.18 | 0.14 | 0.08 | 0.37 | 4552 | 875 | 109 | 9 | 67 |
| 1 | 1 | 1 | 12 | 4.64 | 0.24 | 2.76 | 1.87 | 0.38 | 0.19 | 0.17 | 0.09 | 0.37 | 4474 | 1233 | 140 | 7 | 72 |
| 1 | 1 | 1 | 24 | 4.22 | 0.18 | 2.40 | 1.63 | 0.34 | 0.17 | 0.24 | 0.10 | 0.46 | 4953 | 809 | 183 | 2 | 60 |
| 1 | 1 | 1 | 48 | 5.18 | 0.22 | 2.64 | 1.83 | 0.38 | 0.20 | 0.11 | 0.09 | 0.40 | 5023 | 1215 | 109 | 2 | 52 |
| 1 | 1 | 1 | 96 | 5.37 | 0.25 | 2.76 | 1.80 | 0.39 | 0.23 | 0.16 | 0.08 | 0.43 | 4769 | 510 | 118 | 4 | 58 |
| 2 | 1 | 1 | 12 | 3.92 | 0.16 | 3.05 | 1.45 | 0.38 | 0.17 | 0.19 | 0.09 | 0.45 | 3950 | 608 | 120 | 6 | 59 |
| 2 | 1 | 1 | 24 | 4.70 | 0.18 | 2.77 | 1.58 | 0.42 | 0.21 | 0.20 | 0.08 | 0.37 | 4437 | 1050 | 122 | 5 | 59 |
| 2 | 1 | 1 | 96 | 4.46 | 0.19 | 2.89 | 1.82 | 0.20 | 0.20 | 0.14 | 0.09 | 0.47 | 4367 | 951 | 117 | 4 | 55 |

FUM 0 = non-fumigated, FUM 1 = fumigated, INOC 0 = non-inoculated, INOC 1 = inoculated, TRT = ug P/g.

Table 21: ORIGINAL MEASUREMENTS OF GROWTH VARIABLES OF MYCORRHIZAL AND NON-MYCORRHIZAL COWPEA PLANTS GROWN IN STERILIZED AND UNSTERILIZED SOILS AT FIVE LEVELS OF RESIDUAL PHOSPHATE

| REP | FUM | INOC | TRT | HT1 (cm) | HT2 (cm) | HT3 (cm) | RTW (g/pot) | STW (g/pot) | NOD no. | INF % | PODN (no/plant) | SEEDW (g/plant) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 14 | 25.3 | . | . | 7.60 | 4.30 | 5.5 | 50 | 3 | 2.60 |
| 1 | 0 | 0 | 15 | 25.8 | . | . | 8.90 | 4.80 | 5.0 | 55 | 3 | 1.50 |
| 1 | 0 | 0 | 20 | 28.5 | . | . | 8.90 | 5.90 | 5.0 | 55 | 3 | 2.05 |
| 1 | 0 | 0 | 22 | 26.8 | . | . | 9.10 | 4.00 | 6.5 | 35 | 3 | 2.00 |
| 1 | 0 | 0 | 26 | 33.0 | . | . | 9.60 | 4.60 | 5.5 | 35 | 3 | 2.25 |
| 1 | 0 | 1 | 14 | 24.8 | . | . | 7.80 | 4.10 | 5.0 | 60 | 3 | 1.05 |
| 1 | 0 | 1 | 15 | 27.9 | . | . | 9.40 | 7.20 | 5.0 | 65 | 2 | 1.90 |
| 1 | 0 | 1 | 20 | 27.6 | . | . | 9.20 | 6.60 | 5.0 | 50 | 3 | 1.95 |
| 1 | 0 | 1 | 22 | 30.6 | . | . | 9.50 | 5.30 | 6.0 | 40 | 3 | 2.20 |
| 1 | 0 | 1 | 26 | 31.6 | . | . | 10.10 | 4.80 | 6.0 | 35 | 4 | 1.75 |
| 1 | 1 | 0 | 14 | 14.0 | 16.5 | 14.5 | 1.40 | 1.00 | 2.0 | 0 | 0 | 0.00 |
| 1 | 1 | 0 | 15 | 14.3 | 13.6 | 16.4 | 1.84 | 1.70 | 2.0 | 0 | 0 | 0.00 |
| 1 | 1 | 0 | 20 | 16.4 | 15.0 | 17.0 | 1.70 | 1.55 | 2.5 | 0 | 0 | 0.00 |
| 1 | 1 | 0 | 22 | 17.0 | 15.3 | 16.0 | 1.78 | 2.25 | 4.0 | 0 | 0 | 0.00 |
| 1 | 1 | 0 | 26 | 18.3 | 15.0 | 17.8 | 2.80 | 2.70 | 5.0 | 0 | 0 | 0.00 |
| 1 | 1 | 1 | 14 | 19.0 | 21.0 | 23.0 | 2.75 | 1.93 | 3.0 | 75 | 2 | 2.05 |
| 1 | 1 | 1 | 15 | 18.5 | 13.2 | 32.5 | 4.75 | 2.20 | 3.5 | 75 | 2 | 1.15 |
| 1 | 1 | 1 | 20 | 16.1 | 18.0 | 25.0 | 2.35 | 1.45 | 5.0 | 60 | 2 | 1.12 |
| 1 | 1 | 1 | 22 | 10.5 | 14.8 | 29.8 | 2.20 | 2.38 | 6.0 | 50 | 3 | 1.50 |
| 1 | 1 | 1 | 26 | 18.9 | 19.0 | 30.5 | 2.50 | 2.55 | 6.5 | 40 | 3 | 1.60 |
| 2 | 0 | 0 | 14 | 26.6 | . | . | 8.60 | 5.10 | 4.5 | 50 | 2 | 2.00 |
| 2 | 0 | 0 | 15 | 30.8 | . | . | 9.10 | 5.00 | 5.0 | 55 | 2 | 1.40 |
| 2 | 0 | 0 | 20 | 28.6 | . | . | 9.20 | 5.50 | 5.5 | 55 | 2 | 2.25 |
| 2 | 0 | 0 | 22 | 32.0 | . | . | 9.00 | 7.30 | 6.0 | 35 | 3 | 2.80 |
| 2 | 0 | 0 | 26 | 35.7 | . | . | 10.00 | 8.50 | 6.5 | 30 | 3 | 3.85 |
| 2 | 0 | 1 | 14 | 28.7 | . | . | 8.90 | 7.50 | 5.5 | 60 | 2 | 1.20 |
| 2 | 0 | 1 | 15 | 32.7 | . | . | 9.00 | 4.80 | 5.5 | 65 | 3 | 1.80 |
| 2 | 0 | 1 | 20 | 34.4 | . | . | 9.40 | 5.50 | 6.0 | 50 | 4 | 3.35 |
| 2 | 0 | 1 | 22 | 29.8 | . | . | 9.00 | 7.10 | 6.5 | 40 | 3 | 3.35 |
| 2 | 0 | 1 | 26 | 36.3 | . | . | 10.40 | 11.00 | 7.0 | 35 | 4 | 4.85 |
| 2 | 1 | 0 | 14 | 16.6 | 14.3 | 18.0 | 1.25 | 1.40 | 2.0 | 0 | 0 | 0.00 |
| 2 | 1 | 0 | 15 | 18.4 | 13.6 | 13.5 | 1.33 | 1.20 | 2.0 | 0 | 0 | 0.00 |
| 2 | 1 | 0 | 20 | 15.9 | 14.9 | 15.0 | 1.48 | 1.65 | 2.5 | 0 | 0 | 0.00 |
| 2 | 1 | 0 | 22 | 15.9 | 14.3 | 17.4 | 1.48 | 1.60 | 4.0 | 0 | 0 | 0.00 |
| 2 | 1 | 0 | 26 | 19.3 | 18.5 | 19.8 | 1.50 | 2.45 | 5.0 | 0 | 0 | 0.00 |
| 2 | 1 | 1 | 14 | 17.3 | 20.0 | 26.5 | 1.20 | 1.55 | 3.0 | 75 | 2 | 2.15 |
| 2 | 1 | 1 | 15 | 19.2 | 29.2 | 31.0 | 1.75 | 1.73 | 3.5 | 75 | 2 | 2.10 |
| 2 | 1 | 1 | 20 | 20.5 | 27.4 | 28.4 | 4.03 | 3.50 | 5.0 | 60 | 3 | 1.60 |
| 2 | 1 | 1 | 22 | 19.0 | 20.1 | 29.7 | 3.05 | 3.65 | 6.0 | 60 | 3 | 2.15 |
| 2 | 1 | 1 | 26 | 19.0 | 23.3 | 29.8 | 2.98 | 3.70 | 7.0 | 40 | 3 | 2.20 |
| 3 | 0 | 0 | 14 | 23.4 | . | . | . | . | . | . | . | . |
| 3 | 0 | 0 | 15 | 28.3 | . | . | . | . | . | . | . | . |
| 3 | 0 | 0 | 20 | 27.2 | . | . | . | . | . | . | . | . |
| 3 | 0 | 0 | 22 | 29.9 | . | . | . | . | . | . | . | . |
| 3 | 0 | 0 | 26 | 30.2 | . | . | . | . | . | . | . | . |
| 3 | 0 | 1 | 14 | 26.5 | . | . | . | . | . | . | . | . |
| 3 | 0 | 1 | 15 | 30.3 | . | . | . | . | . | . | . | . |
| 3 | 0 | 1 | 20 | 28.5 | . | . | . | . | . | . | . | . |
| 3 | 0 | 1 | 22 | 30.2 | . | . | . | . | . | . | . | . |
| 3 | 0 | 1 | 26 | 28.3 | . | . | . | . | . | . | . | . |
| 3 | 1 | 0 | 14 | 16.0 | . | 16.9 | . | . | . | 0 | 0 | 0.00 |
| 3 | 1 | 0 | 15 | 16.4 | . | 11.7 | . | . | . | 0 | 0 | 0.00 |
| 3 | 1 | 0 | 20 | 17.1 | . | 16.0 | . | . | . | 0 | 0 | 0.00 |
| 3 | 1 | 0 | 22 | 15.8 | . | 12.0 | . | . | . | 0 | 0 | 0.00 |
| 3 | 1 | 0 | 26 | 18.5 | . | 20.8 | . | . | . | 0 | 0 | 0.00 |
| 3 | 1 | 1 | 14 | 19.4 | . | 14.0 | . | . | . | 75 | 3 | 2.60 |
| 3 | 1 | 1 | 15 | 18.0 | . | 20.1 | . | . | . | 75 | 2 | 1.63 |
| 3 | 1 | 1 | 20 | 15.0 | . | 28.3 | . | . | . | 60 | 4 | 2.40 |
| 3 | 1 | 1 | 22 | 19.4 | . | 30.0 | . | . | . | 60 | 2 | 2.45 |
| 3 | 1 | 1 | 26 | 16.8 | . | 29.0 | . | . | . | 40 | 3 | 3.20 |
| 4 | 1 | 0 | 14 | 15.8 | . | 10.8 | . | . | . | 0 | 0 | 0.00 |
| 4 | 1 | 0 | 15 | 14.4 | . | 13.0 | . | . | . | 0 | 0 | 0.00 |
| 4 | 1 | 0 | 20 | 15.3 | . | 14.8 | . | . | . | 0 | 0 | 0.00 |
| 4 | 1 | 0 | 22 | 14.6 | . | 14.0 | . | . | . | 0 | 0 | 0.00 |
| 4 | 1 | 0 | 26 | 19.5 | . | 17.0 | . | . | . | 0 | 0 | 0.00 |
| 4 | 1 | 1 | 14 | 17.7 | . | 22.0 | . | . | . | 75 | 2 | 1.90 |
| 4 | 1 | 1 | 15 | 13.5 | . | 19.5 | . | . | . | 75 | 2 | 1.65 |
| 4 | 1 | 1 | 20 | 16.1 | . | 42.0 | . | . | . | 60 | 2 | 1.05 |
| 4 | 1 | 1 | 22 | 17.1 | . | 29.3 | . | . | . | 50 | 3 | 2.00 |
| 4 | 1 | 1 | 26 | 18.3 | . | 29.8 | . | . | . | 40 | 4 | 2.40 |

Table 22: NUTRIENT ANALYSES OF LEAVES OF MYCORRHIZAL AND NON-MYCORRHIZAL COWPEA PLANTS SAMPLED AT 50% FLOWERING AND GROWN IN STERILIZED AND UNSTERILIZED SOILS AT FIVE LEVELS OF RESIDUAL PHOSPHATE

| | | | | percent | | | | | | | | ppm | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| REP | FUM | INOC | TRT | N | P | K | CA | MG | S | SI | NA | CL | AL | MN | FE | CU | ZN |
| 1 | 0 | 0 | 14 | 2.72 | 0.12 | 2.38 | 1.11 | 0.26 | 0.13 | 0.10 | 0.08 | 0.44 | 287 | 285 | 94 | 9 | 50 |
| 1 | 0 | 0 | 15 | 2.39 | 0.12 | 1.68 | 1.02 | 0.25 | 0.12 | 0.08 | 0.08 | 0.57 | 329 | 197 | 99 | 7 | 45 |
| 1 | 0 | 0 | 20 | 2.19 | 0.11 | 1.73 | 1.77 | 0.32 | 0.13 | 0.18 | 0.09 | 0.66 | 669 | 443 | 108 | 4 | 45 |
| 1 | 0 | 0 | 22 | 2.26 | 0.11 | 1.76 | 1.43 | 0.30 | 0.14 | 0.10 | 0.08 | 0.54 | 276 | 230 | 90 | 4 | 45 |
| 1 | 0 | 0 | 26 | 2.41 | 0.13 | 2.15 | 2.01 | 0.30 | 0.16 | 0.15 | 0.08 | 0.78 | 1468 | 379 | 91 | 3 | 50 |
| 1 | 0 | 1 | 14 | 2.73 | 0.14 | 2.00 | 1.68 | 0.37 | 0.16 | 0.18 | 0.09 | 0.57 | 368 | 308 | 97 | 7 | 60 |
| 1 | 0 | 1 | 15 | 2.59 | 0.12 | 2.01 | 1.57 | 0.32 | 0.16 | 0.15 | 0.08 | 0.74 | 675 | 275 | 92 | 6 | 53 |
| 1 | 0 | 1 | 20 | 2.89 | 0.15 | 1.97 | 1.73 | 0.39 | 0.18 | 0.15 | 0.08 | 0.77 | 313 | 252 | 108 | 5 | 56 |
| 1 | 0 | 1 | 22 | 1.86 | 0.11 | 1.89 | 2.01 | 0.33 | 0.13 | 0.19 | 0.08 | 0.66 | 923 | 387 | 107 | 4 | 61 |
| 1 | 0 | 1 | 26 | 1.64 | 0.10 | 1.30 | 1.02 | 0.27 | 0.10 | 0.13 | 0.08 | 0.37 | 410 | 267 | 98 | 3 | 37 |
| 2 | 0 | 0 | 14 | 2.69 | 0.11 | 1.89 | 1.50 | 0.38 | 0.14 | 0.18 | 0.07 | 0.56 | 1248 | 323 | 117 | 4 | 50 |
| 2 | 0 | 0 | 15 | 2.03 | 0.10 | 1.51 | 1.09 | 0.21 | 0.12 | 0.09 | 0.08 | 0.53 | 2401 | 214 | 90 | 4 | 43 |
| 2 | 0 | 0 | 20 | 2.71 | 0.13 | 2.08 | 2.29 | 0.32 | 0.17 | 0.27 | 0.08 | 0.64 | 1613 | 411 | 105 | 4 | 70 |
| 2 | 0 | 0 | 22 | 2.26 | 0.11 | 1.76 | 1.43 | 0.30 | 0.14 | 0.10 | 0.08 | 0.54 | 276 | 230 | 90 | 4 | 45 |
| 2 | 0 | 0 | 26 | 2.68 | 0.12 | 1.74 | 1.59 | 0.30 | 0.15 | 0.10 | 0.08 | 0.72 | 458 | 284 | 100 | 6 | 49 |
| 2 | 0 | 1 | 14 | 2.73 | 0.14 | 2.00 | 1.68 | 0.37 | 0.16 | 0.18 | 0.09 | 0.57 | 368 | 308 | 97 | 7 | 60 |
| 2 | 0 | 1 | 15 | 2.59 | 0.12 | 2.01 | 1.57 | 0.32 | 0.16 | 0.15 | 0.08 | 0.74 | 675 | 275 | 92 | 6 | 53 |
| 2 | 0 | 1 | 20 | 2.89 | 0.15 | 1.97 | 1.73 | 0.39 | 0.18 | 0.15 | 0.08 | 0.77 | 313 | 252 | 108 | 5 | 56 |
| 2 | 0 | 1 | 22 | 1.86 | 0.11 | 1.89 | 2.01 | 0.33 | 0.13 | 0.19 | 0.08 | 0.66 | 923 | 387 | 107 | 4 | 61 |
| 2 | 0 | 1 | 26 | 3.08 | 0.16 | 2.30 | 2.46 | 0.37 | 0.20 | 0.21 | 0.08 | 0.65 | 1062 | 308 | 99 | 5 | 56 |
| 1 | 1 | 0 | 22 | 2.73 | 0.11 | 1.87 | 1.19 | 0.32 | 0.11 | 0.15 | 0.08 | 0.47 | 4554 | 943 | 94 | 1 | 60 |
| 1 | 1 | 1 | 14 | 3.90 | 0.19 | 2.65 | 1.78 | 0.42 | 0.19 | 0.23 | 0.09 | 0.38 | 4075 | 275 | 103 | 16 | 71 |
| 1 | 1 | 1 | 15 | 3.31 | 0.14 | 2.23 | 1.40 | 0.39 | 0.15 | 0.16 | 0.08 | 0.36 | 4144 | 496 | 104 | 4 | 63 |
| 1 | 1 | 1 | 20 | 3.90 | 0.17 | 3.09 | 1.86 | 0.34 | 0.19 | 0.29 | 0.08 | 0.48 | 4278 | 468 | 111 | 8 | 74 |
| 1 | 1 | 1 | 22 | 4.05 | 0.20 | 2.36 | 1.96 | 0.35 | 0.18 | 0.19 | 0.09 | 0.45 | 5359 | 347 | 115 | 5 | 77 |
| 1 | 1 | 1 | 26 | 3.62 | 0.14 | 2.28 | 1.71 | 0.36 | 0.16 | 0.16 | 0.08 | 0.54 | 5249 | 555 | 101 | 1 | 66 |
| 2 | 1 | 1 | 22 | 4.12 | 0.18 | 2.95 | 1.87 | 0.40 | 0.18 | 0.23 | 0.08 | 0.43 | 4604 | 361 | 112 | 7 | 73 |
| 2 | 1 | 1 | 26 | 4.38 | 0.21 | 2.93 | 1.52 | 0.31 | 0.18 | 0.12 | 0.08 | 0.41 | 3045 | 359 | 101 | 8 | 60 |

FUM 0 = non-fumigated, FUM 1 = fumigated, INOC 0 = non-inoculated, INOC 1 = inoculated, TRT = ug P/g.

Table 23: Analysis of variance table for the effect of methyl bromide fumigation and inoculation with *Glomus mosseae* on the growth of cowpea in soil with newly applied phosphate.

| Source of Variation | Degrees of freedom | Mean Squares HT1 | HT2 | SEEDW | PODN | INF |
|---|---|---|---|---|---|---|
| REP | 3 | 345.85** | 264.38** | 7.39** | 9.32** | 0.04** |
| FUM | 1 | 3700.49** | -+ | 80.32** | 72.90** | 0.38** |
| INOC | 1 | 5.38 | 278.78** | 47.09** | 28.02** | 3.55** |
| FUMxINOC | 1 | 0.52 | -+ | 8.49** | 20.83** | 1.09** |
| P | 4 | 68.73** | 87.64* | 8.74** | 7.93** | 0.11** |
| FUMxP | 4 | 0.97 | -+ | 0.72 | 0.68 | 0.02** |
| INOCxP | 4 | 1.17 | 2.68 | 0.94 | 0.27 | 0.04** |
| FUMxINOCxP | 4 | 4.12 | -+ | 0.13 | 0.89 | 0.02** |
| ERROR | - | 7.49 | 24.83 | 0.49 | 0.29 | 0.0003 |
| ERROR DF | | 43++ | 23+++ | 37 | 37 | 37 |
| C.V. | | 11.1% | 22.7% | 28.8% | 23% | 4.5% |

* = Significant at the 5% level of probability

** = Significant at the 1% level of probability

\+ = No measurements. Plants in non-fumigated treatments were harvested before this date.

++ = 4 DF lost due to missing plots

+++ = 2 DF lost due to missing plots.

Table 23: (continued) Analysis of variance table for the effect of methyl bromide fumigation and inoculation with *Glomus mosseae* on the growth of cowpea in soil with newly applied phosphate.

| Source of Variation | Degrees of freedom | Mean Squares | | |
|---|---|---|---|---|
| | | HT3 | RTW | NOD |
| REP | 1 | 39.76 | 2.49 | 0.03 |
| FUM | 1 | -+ | 44.66** | 75.63** |
| INOC | 1 | 1524.26** | 2.19 | 5.63** |
| FUMxINOC | 1 | -+ | 0.003 | 4.23** |
| P | 4 | 69.69 | 4.49** | 13.48** |
| FUMxP | 4 | -+ | 0.38 | 0.39** |
| INOCxP | 4 | 27.99 | 0.12 | 0.58** |
| FUMxINOCxP | 4 | -+ | 0.25 | 0.62** |
| ERROR | - | 35.64 | 0.92 | 0.04 |
| ERROR DF | | 9 | 19 | 19 |
| C.V. | | 21.9% | 29% | 3.6% |

* = Significant at the 5% level of probability

** = Significant at the 1% level of probability

\+ = No measurements. Plants in non-fumigated treatments were harvested before this date.

Table 24: Analysis of variance table for the effect of methyl bromide fumigation and inoculation with *Glomus mosseae* on the growth of cowpea in soil with residual phosphate.

| Source of Variation | Degrees of freedom | Mean Squares | | | |
|---|---|---|---|---|---|
| | | HT1 | HT3 | SEEDW | PODW |
| REP | 3 | 167.37** | 17.46 | 2.35** | 2.36** |
| FUM | 1 | 2207.05** | -+ | 20.28** | 27.23** |
| INOC | 1 | 19.35* | 1413.72** | 26.00** | 52.27** |
| FUMxINOC | 1 | 0.04 | -+ | 11.66** | 16.13** |
| P | 4 | 27.11** | 51.55* | 0.98** | 1.08** |
| FUMxP | 4 | 14.21** | -+ | 0.88** | 0.92 |
| INOCxP | 4 | 4.38 | 25.92 | 0.18 | 0.68 |
| FUMxINOCxP | 4 | 0.74 | -+ | 0.55 | 0.09 |
| ERROR | - | 3.71 | 15.52 | 0.23 | 0.21 |
| ERROR DF | - | 43++ | 27 | 37 | 37 |
| C.V. | | 8.3% | 18.3% | 33.7% | 25% |

* = Significant at the 5% level of probability

** = Significant at the 1% level of probability

\+ = No measurements. Plants in non-fumigated treatments were harvested before this date.

++ = 4 DF lost due to missing plots.

Table 24: (continued) Analysis of variance table for the affect of methyl bromide fumigation and inoculaiton with VA mycorrhiza on the growth of cowpea in soil with residual phosphate.

| Source of Variation | Degrees of freedom | Mean Squares | | | |
|---|---|---|---|---|---|
| | | HT2 | RTW | STW | NOD |
| REP | 1 | 29.28 | 0.06 | 8.48 | 0.40 |
| FUM | 1 | -+ | 480.53** | 147.30* | 27.25** |
| INOC | 1 | 296.45 | 4.73 | 6.43 | 10.00** |
| FUMxINOC | 1 | -+ | 1.75 | 0.08 | 5.63** |
| P | 4 | 8.77 | 1.75* | 3.40 | 8.10** |
| FUMxP | 4 | -+ | 0.31 | 0.22 | 1.63** |
| INOCxP | 4 | 21.07 | 0.11 | 0.03 | 0.16 |
| FUMxINOCxD | 4 | -+ | 0.17 | 0.21 | 0.19 |
| ERROR | - | 10.84 | 0.53 | 2.31 | 0.124 |
| ERROR DF | | 9 | 19 | 17++ | 19 |
| C.V. | | 17.4% | 12.9% | 35.7% | 7.3% |

* = Significant at the 5% level of probability

** = Significant at the 1% level of probability

\+ = No measurements. Plants in non-fumigated treatments were harvested before this date.

++ = 2 DF lost due to missing plots.

Table 25: Comparison of means of growth variables and nutrient concentrations in leaves of cowpea plants grown at 5 dilutions of non-sterile and sterile soil.

| Dilution | Growth Variables | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | HT am | RTW g/pot | STW g/pot | N % | P % | S % | INF % | MN ppm |
| 1/0 | 13.6 | 2.2 | 2.3 | 1.97b | 0.11 | 0.15 | 29.4a | 271b |
| 1/4 | 15.5 | 2.3 | 2.5 | 2.23ba | 0.11 | 0.15b | 22.4b | 284b |
| 1/16 | 15.7 | 2.3 | 2.6 | 2.51a | 0.11 | 0.19a | 12.5c | 336a |
| 1/64 | 14.6 | 2.4 | 2.5 | 2.57a | 0.11 | 0.21a | 6.5d | 337a |
| 1/256 | 14.9 | 2.3 | 2.6 | 2.39ba | 0.11 | 0.20a | 3.6e | 373a |

Means followed by the same letter are not significantly different at the 5% level.

Table 26: Comparison of mean of growth variables of cowpea in fumigated and non-fumigated soils with newly applied and residual phosphate.

| Soil Fumigation | | Growth Variables | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | HT1 cm | RTW g/ plant | STW g/ plant | NOD no/ plant | POD YLD no/ plant | SEED YLD g/ plant | INF % |
| New P | - | 32.44** | 4.38** | 15.63** | 6.85** | 4.15** | 4.28** | 53** |
| | + | 16.71 | 2.24 | 3.10 | 4.10 | 1.45 | 1.51 | 34 |
| Res. P | - | 29.36** | 9.14** | 5.95** | 5.63** | 2.90 | 2.31** | 50** |
| | + | 16.98 | 2.20 | 2.10 | 3.97 | 1.30 | 0.97 | 33 |

**Difference between mean for ± Fumigation significant at $P < 0.01$.

Table 27: Comparison of means of growth variables of cowpea plants which were inoculated with *Glomus mosseae* and grown in soils with freshly applied and residual phosphate.

| Soil | Inoc. | Growth Variables | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | HT1 cm | HT2 cm | HT3 cm | RTW g/ plant | STW g/ plant | NOD no/ plant | POD no/ plant | SEED g/ plant | INF % |
| New P | - | 23.2 | 18.4 | 18.4 | 3.07 | 7.19 | 5.10 | 1.67 | 1.54 | 16 |
| | + | 23.7 | 23.7** | 35.9** | 3.54 | 9.03 | 5.88** | 3.03** | 3.31** | 65** |
| Res. P | - | 21.7 | 15.1 | 15.6 | 5.34 | 3.63 | 4.30 | 0.90 | 0.76 | 16 |
| | + | 22.8* | 22.8** | 27.5** | 6.01** | 4.43 | 5.30** | 2.77** | 2.07** | 62** |

**Difference between means for ± inoculation significant at $P < 0.01$.
* Difference between means for ± inoculation significant at $P < 0.05$.

Table 28: Comparison of means of growth variables of cowpea plants grown in soils with 5 levels of newly applied and residual phosphate.

| Soil | P Level µg/g | Growth Variables | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | HT1 cm | HT2 cm | HT3 cm | RTW g/ plant | STW g/ plant | NOD no/ plant | PODN no/ plant | SEEDW g/ plant | INF % |
| Newly Applied P | 0 | 21.2b | 17.7b | 20.8b | 2.09b | 7.06a | 3.75e | 1.42d | 1.58d | 48a |
| | 12 | 21.9b | 17.6b | 22.9ba | 3.20a | 8.02a | 4.69d | 1.92c | 1.86d | 49a |
| | 24 | 22.6b | 21.7ba | 29.9ba | 3.56a | 8.13a | 5.50c | 2.25b | 2.36cb | 42b |
| | 48 | 25.5a | 24.1a | 30.7a | 3.57a | 8.32a | 6.56b | 2.58b | 2.55b | 34c |
| | 96 | 26.1a | 24.3a | 31.4a | 4.09a | 9.04a | 6.88a | 3.58a | 3.79a | 27d |
| Residual P | 14 | 20.8b | 17.9a | 18.2c | 4.94b | 3.36b | 3.81d | 1.58bc | 1.29bc | 48b |
| | 15 | 21.9b | 21.4a | 19.7c | 5.64ab | 3.58ab | 3.94d | 1.50c | 1.09c | 49a |
| | 20 | 22.0b | 18.3a | 22.3bac | 5.76ab | 3.96ab | 4.56c | 1.92ba | 1.31bc | 39c |
| | 22 | 22.1b | 17.6a | 23.3ba | 5.78a | 4.19ab | 3.63b | 1.92ba | 1.54ba | 32d |
| | 26 | 24.6a | 18.9a | 24.3a | 6.24a | 5.04a | 6.06a | 2.25a | 1.84a | 25e |

Means followed by the same letter are not significantly different at the 5% level.

Table 29: Comparison of means of nutrient concentrations in leaves of cowpea plants which were inoculated with Glomus mosseae and growth in soils with newly applied and residual phosphate.

| Soil | Inoculation | N | P | K | Ca | Mg | S | Si | Na | Cl | Al | Mn | Fe | Cu | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Percent | | | | | | | | | ppm | | | | |
| Newly Applied P | - | 2.82 | 0.13 | 2.13 | 1.81 | 0.27 | 0.16 | 0.15 | 0.08 | 0.60* | 1075 | 500 | 99 | 5 | 54 |
| | + | 3.47* | 0.16* | 2.22 | 1.62 | 0.29 | 0.16 | 0.14 | 0.08 | 0.49 | 2772* | 589 | 109* | 7 | 51 |
| Residual P | - | 2.46 | 0.11 | 1.86 | 1.49 | 0.29 | 0.13 | 0.13 | 0.08 | 0.58 | 1234 | 358 | 98 | 5 | 50 |
| | + | 3.07* | 0.15 | 2.22 | 1.74 | 0.35* | 0.16* | 0.18* | 0.08 | 0.56 | 2163* | 346 | 103* | 6 | 61* |

*Difference between means of ± inoculation significant at $P < 0.05$.

Table 30: Comparison of means of nutrient concentrations in leaves of cowpea plants grown in fumigated and non-fumigated soils with newly applied and residual phosphate.

| Soil | Fumi-gation | N | P | K | Ca | Mg | S | Si | Na | Cl | Al | Mn | Fe | Cu | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Percent | | | | | | | | | ppm | | | | |
| Newly Applied P | - | 2.61 | 0.12 | 1.92 | 1.69 | 0.23 | 0.15 | 0.13 | 0.08 | 0.40 | 1098 | 354 | 98 | 5 | 49 |
| | + | 4.59* | 0.20* | 2.77* | 1.69 | 0.38* | 0.19* | 0.17* | 0.09 | 0.59* | 4417* | 1002* | 125* | 5 | 59 |
| Residual P | - | 2.46 | 0.12 | 1.90 | 1.64 | 0.32 | 0.15 | 0.15 | 0.08 | 0.44 | 753 | 301 | 99 | 5 | 52 |
| | + | 3.75* | 0.17* | 2.55* | 1.66 | 0.36* | 0.17 | 0.19* | 0.08 | 0.62* | 4413* | 475* | 105* | 6 | 68* |

*Difference between means of ± fumigation significant at $P < 0.05$.

## LITERATURE CITED


1. Abbott, K. L., and K. D. Robson. 1977. Growth Stimulation of Subterranean clover with Vesicular-arbuscular mycorrhizas. Aust. J. Agric. Res. 28:639-649.

2. Aldrich, D. G., and J. P. Martin. 1952. Effect of fumigation on some chemical properties of soils. Soil Sci. 73:149-159.

3. Altman, J. 1970. Increase and decrease in plant growth response resulting from soil fumigation. p. 216-221. In T. A. Tousson, R. V. Bega and P. E. Nelson (eds) Root diseases and soil-borne pathogens. Univ. of California, Berkeley.

4. Altman, J., and K. M. Tsue. 1965. Changes in plant growth with chemicals used as soil fumigants. Plant Dis. Rept. 49:600-602.

5. Andrew, C. S., and M. F. Robins. 1969. The effect of phosphorus on the growth and chemical composition of some tropical legumes: I. Growth and critical percentages of phosphorus. Aust. J. Agric. Res. 20: 665-674.

6. Bagyaraj, D. J., A. Manjunath, and R. B. Patil. 1979a. Interaction between a vesicular-arbuscular mycorrhiza and Rhizobium and their effects on soybean in the field. New Phytol. 82:141-145.

7. Bagyaraj, D. J., A. Manjunath, and R. B. Patil. 1979b. Occurrence of vesicular-arbuscular mycorrhizas in some tropical aquatic plants. Trans. Brit. Mycol. Soc. 72:164-167.

8. Baker, R. 1965. The dynamics of Inoculum. p. 395-403, 415-419. In K. F. Baker and W. C. Snyder (eds) Ecology of soil-borne plant pathogens. Univ. of California, Berkeley.

9. Baylis, G. T. S. 1967. Experiments on the ecological significance of Phycomycetous mycorrhizas. New Phytol. 66:231-243.

10. Baylis, G. T. S. 1959. Effect of vesicular-arbuscular mycorrhiza on growth of Griselinia littoralis (Cornaceae). New Phytol. 58:274-280.

11. Baylis, G. T. S. 1972. Minimum levels of available phosphorus for non-mycorrhizal plants. Plant and Soil. 36:233-234.

12. Beauverie, J. 1902. Etude d'une hepatique à thalle habité par un Champignon filamentaux. Compt. Rend. Acad. Sc. Paris. 134:616-618.

13. Becker, W. N., and J. W. Gerdemann. 1977. Glomus etunicatus sp. nov. Mycotaxon. 6:29-32.

14. Bergeson, F. J. 1971. Biochemistry of symbiotic nitrogen fixation in legumes. Ann. Rev. Plant Physiol. 22:121-140.

15. Bhat, K. S., and P. H. Nye. 1974. Diffusion of phosphate to plant roots in soil: III. Depletion zones around onion roots without root hairs. Plant and Soil. 41: 383-394.

16. Bieleski, R. L. 1973. Phosphate pools, phosphate transport and phosphate availability. Ann. Rev. Plant Physiol. 24:225-252.

17. Biermann, B., and R. G. Linderman. 1981. Quantifying vesicular-arbuscular mycorrhizae: A proposed method towards standardization. New Phytol. 87:63-67.

18. Black, R. L. B., and P. B. Tinker. 1977. Interaction between effects of vesicular-arbuscular mycorrhiza and fertilizer phosphorus on yield of potatoes in the field. Nature (London) 267:510-511.

19. Borea, J. M., M. E. Brown, and B. Mosse. 1972. Association between VA mycorrhiza and Azotobacter. Rothamsted Exp. Stn. Rep., part 1, p. 81.

20. Butler, E. J. 1939. The occurrence and systematic position of vesicular-arbuscular type of mycorrhizal fungus. Trans. Brit. Mycol. Soc. 22:274-301.

21. Carling, D. E., M. F. Brown, and R. A. Brown. 1979a. Colonization rates and growth responses of soybean plants infected by vesicular-arbuscular mycorrhizal fungi. Can. J. Bot. 57:1769-1772.

22. Carling, D. E., M. F. Brown, and R. A. Brown. 1979b. Effects of vesicular-arbuscular mycorrhizal fungus on nitrate reductase and nitrogenase activity on nodulating and non-nodulating soybeans. Phytopath. 68:1590-1596.

23. Ciferri, R. 1928. Preliminary observation of sugarcane mycorrhiza and their relationship to root diseases. Phytopath. 18:249-261.

24. Cooper, K. M. 1975. Growth response to the formation of endotrophic mycorrhiza in Solanum leptospernum and New Zealand ferns. p. 391-407. In F. E. Sanders, B. Mosse and P. B. Tinker (eds) Endomycorrhizas. Academic Press, London.

25. Cooper, K. M., and P. B. Tinker. 1978. Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. II. Uptake and translocation of phosphorus, zinc and sulphur. New Phytol. 81:43-52.

26. Crush, J. R. 1974. Plant growth response to vesicular-arbuscular mycorrhiza. VII. Growth and nodulation of some herbage legumes. New Phytol. 73:743-749.

27. Crush, J. R. 1976. Endomycorrhizas and legume growth in some soils of the MacKenzie Basin, Cantebury, New Zealand. N. Z. J. Agric. Res. 19:473-476.

28. Cox, G., and F. Sanders. 1974. Ultrastructure of the host-fungus interface in vesicular-arbuscular mycorrhiza. New Phytol. 73:900-912.

29. Daft, M. F. and A. A. El-Giahmi. 1976. Studies on nodulated and mycorrhizal peanuts. Ann. Appl. Biol. 83:273-276.

30. Daft, M. F., E. Hacskaylo, and T. H. Nicolson. 1975. Arbuscular mycorrhizas in plants colonising coal spoils in Scotland and Pennsylvania. p.561-58 . In F. E. Sanders, B. Mosse, and P. B. Tinker (eds) Endomycorrhizas. Academic Press, London.

31. Daft M. J., and T. H. Nicolson. 1966. Effect of Endogone mycorrhiza on plant growth. New Phytol. 65: 343-350.

32. Daft M. J., and T. H. Nicolson. 1969. Effect of Endogone mycorrhiza on plant growth. III. Influence of inoculum concentration on growth and infection of tomato. New Phytol. 68:953-961.

33. Daft M. J., and T. H. Nicolson. 1972. Effect of Endogone mycorrhiza on plant growth. IV. Quantitative relationship between the growth of the host and the development of the endophyte in tomato and maize. New Phytol. 71:287-295.

34. Daft M. J., and B. O. Okusanya. 1973. Effect of Endogone mycorrhiza on plant growth. IV. Influence of infection on the anatomy and reproductive development in four hosts. New Phytol. 72:1333-1339.

35. Davey, C. B., and R. M. Danielson. 1968. Soil chemical factors and biological activity. Phytopath. 58:900-908.

36. Dehne, R. M., and F. Schonbeck. 1975. The influence of the endotrophic mycorrhiza on the fusarial wilt of tomato. Z. Pflanzenkr and Pflanzenschutz. 82:630-632.

37. Dowding, E. S. 1959. Ecology of Endogone. Trans. Brit. Mycol. Soc. 42:449-457.

38. Dowdle, S. F. 1980. Evaluating mycorrhizal inoculum levels in soil and quantifying their contribution to the phosphorus nutrition of cowpea. M.S. thesis, Dept. of Agronomy and Soil Science. University of Hawaii.

39. Eno, C., and H. Popenoe. 1963. The effect of gamma irradiation on the availability of nitrogen and phosphorus in soil. Soil Sci. Soc. Amer. Proc. 27: 299-301.

40. Eno, C., and H. Popenoe. 1964. Gamma irradiation compared with steam and methyl bromide as a soil sterilizing agent. Soil Sci. Soc. Amer. Proc. 28: 533-535.

41. Frank, A. B. 1885. Ueber die auf Wurzelsymbiose beruhende Ernahrung gewisser Baume durch unterirdische Pilze. Ber. Deut. Bot. Gesell. 3:128-145.

42. Furlan, V., and J. A. Fortin. 1973. Formation of endomycorrhizas by Endogone colospora on Allium cepa under three temperature regimes. Naturaliste Can. 100:467-477.

43. Gerdemann, J. W. 1955. Relation of a large soil-borne spore to Phycomycetous mycorrhizal infections. Mycologia. 47:619-632.

44. Gerdemann, J. W. 1964. Effect of mycorrhiza on the growth of maize. Mycologia. 56:342-349.

45. Gerdemann, J. W. 1968. Vesicular-arbuscular mycorrhiza and plant growth. Ann. Rev. Phytopath. 6:397-417.

46. Gerdemann, J. W. 1975. Vesicular-arbuscular mycorrhizae p. 575-591. In J. G. Torrey and D. T. Clarkson (eds) The development and function of roots. Academic Press, London.

47. Gerdemann, J. W., and B. K. Bakshi. 1976. Endogonaceae in India: Two new species. Trans. Brit. Mycol. Soc. 66:340-343.

48. Gerdemann, J. W., and T. H. Nicolson. 1962. Endogone spores in cultivated soils. Nature (London) 195:308.

49. Gerdemann, J. W., and T. H. Nicolson. 1963. Spores of mycorrhizal Endogone species extracted from soil by wet-sieving and decanting. Trans. Brit. Mycol. Soc. 46:235-246.

50. Gerdemann, J. W., and J. M. Trappe. 1975. Taxonomy of the Endogonaceae. p. 35-51. In F. E. Sanders, B. Mosse,and P. B. Tinker (eds) Endomycorrhizas. Academic Press, London.

51. Gerdemann, J. W., and J. M. Trappe. 1975. Taxonomy of the Endogonaceae. p. 35-51. In F. E. Sanders, B. Mosse, and P. B. Tinker (eds). Endomycorrhizas. Academic Press. N. Y.

52. Gerretsen, F. C. 1937. Manganese deficiency of oats and its relation to soil bacteria. Ann. Bot. (London) 1:207-230.

53. Gerretsen, F. C. 1948. The influence of on the phosphate uptake by plants. Plant and Soil 1:51-81.

54. Gilmore, A. E. 1971. The influence of endotrophic mycorrhizae on the growth of peach seedlings. J. Amer. Soc. Hort. Sci. 96:35-38.

55. Ginsburg, O., and Z. Avizohar Hershensen. 1965. Observation on vesicular-arbuscular mycorrhiza associated with avocado roots in Israel. Trans. Brit. Mycol. Soc. 48:101-104.

56. Gray, L. E., and J. W. Gerdemann. 1973. Uptake of sulphur-35 by vesicular-arbuscular mycorrhizae. Plant and Soil. 39:687-689.

57. Hayman, D. S. 1970. Endogone spore numbers in soil and vesicular-arbuscular mycorrhiza in wheat as influenced by season and soil treatment. Trans. Brit. Mycol. Soc. 54:53-63.

58. Hayman, D. S. 1975. The occurrence of mycorrhiza in crops as affected by soil fertility. p. 495-509. In F. E. Sanders, B. Mosse, and P. B. Tinker (eds) Endomycorrhizas. Academic Press, London.

59. Hayman, D. S., and B. Mosse. 1971. Plant growth response to vesicular-arbuscular mycorrhiza: I. Growth of Endogone-inoculated plants in phosphate deficient soils. New Phytol. 70:19-27.

60. Hayman, D. S., and B. Mosse. 1972. Plant growth response to vesicular-arbuscular mycorrhiza: III. Increased uptake of labile P from soil. New Phytol. 71:41-47.

61. Hayman, D. S., and G. E. Stovold. 1979. Spore population and infectivity of vesicular-arbuscular mycorrhizal fungi in New South Wales. Aust. J. Bot. 27:227-233.

62. Hall, I. R., and B. J. Fish. 1979. A key to the Endogonaceae. Trans. Brit. Mycol. Soc. 73:261-270.

63. Ham, A. M. 1962. Studies on vesicular-arbuscular Endophytes. IV. Inoculation of species of Allium and Lactuca sativa with Pythium isolates. Trans. Brit. Mycol. Soc. 45:179-199.

64. Harley, J. L. 1950. Recent progress in the study of endotrophic mycorrhiza. New Phytol. 49:213-247.

65. Harley, J. L. 1969. The biology of mycorrhiza. 2nd ed., Leonard Hill, London.

66. Hattingh, M. J., L. E. Gray, and J. W. Gerdemann. 1973. Uptake and translocation of P-32 labelled phosphate to onion roots by mycorrhizal fungi. Soil Science. 116:383-387.

67. Hattingh, M. J. 1975. Uptake of $^{32}P$ labelled phosphate by endomycorrhizal roots in soil chambers. p. 289-295. In F. E. Sanders, B. Mosse, and P. B. Tinker (eds) Endomycorrhizas. Academic Press, London.

68. Holevas, C. D. 1966. The effect of a vesicular-arbuscular mycorrhiza on the uptake of phosphorus by strawberry (Fragaria sp., var. Cambridge Favorite). J. Hort. Sci. 41:57-64.

69. Johnson, A. 1969. Vesicular-arbuscular mycorrhiza in sea island cotton and other tropical plants. Trop. Agric. (Trinidad) 26:118-121.

70. Jones, F. R. 1924. A mycorrhizal fungus in the roots of legumes and some other plants. J. Agric. Res. 29: 459-470.

71. Katenin, A. 1963. Endotrophic mycorrhiza of species of Liliaceae and Ranunculaceae in the arctic. Rev. appl. mycol. 42:98.

72. Kelly, A. P. 1931. The concept of mycorrhiza. Mycologia. 23:147-151.

73. Khan, A. G. 1972. The effect of vesicular-arbuscular mycorrhizal association on growth of cereals: I. Effect on maize growth. New Phytol. 71:613-619.

74. Khan, A. G. 1975. The effect of vesicular-arbuscular mycorrhizal association on growth of cereals: II. Effect on wheat growth. Ann. Appl. Biol. 80:27-36.

75. Kleinschmidt, C. D., and J. W. Gerdemann. 1972. Stunting of citrus seedlings in fumigated nursery soils related to absence of endomycorrhizae. Phytopath. 62:1447-1453.

76. Kormanik, P. P., W. C. Bryan, and K. C. Schultz. 1977. Influence of Endomycorrhizae on growth of sweetgum seedlings from eight mother trees. For. Sci. 23:500-505.

77. Kormanik, P. P., and A. C. McGraw. 1982. Quantification of vesicular-arbuscular mycorrhiza in plant roots. p.37-45. In N. C. Schenck (ed) Methods and Principles of mycorrhizal research. Amer. Phytopath. Soc. Minnesota.

78. Kreutzer, W. A. 1960. Soil Treatment. p. 431-476. In J. G. Horsefall, and A. E. Dimond (eds) Plant Pathology vol. III. Academic Press, New York.

79. Laycock, D. H. 1945. Preliminary investigation into the function of the endotrophic mycorrhiza of Theobroma cacao L. Trop. Agric. (Trinidad) 22:77-80.

80. Lanowski, J. 1966. Effect of different sources of nitrogen on the development of mycorrhiza in Pisum satirum. Pam Pulawski. 21:365-386.

81. Lewis, D. H. 1973. Concepts in fungal nutrition and the origin of biotrophy. Biol. Rev. 48:261-278.

82. Linderman, R. G., and J. W. Hendrix. 1982. Evaluation of plant response to colonization by vesicular-arbuscular mycorrhizal fungi. p. 69-76. In N. C. Schenck (ed) Methods and Principles of Mycorrhizal Research. Amer. Phytopath. Soc. Minnesota.

83. Ling-lee, M., G. A. Chilvers, and A. E. Ashford. 1975. Polyphosphate granules in three different kinds of tree mycorrhiza. New Phytol. 755:551-554.

84. Lopes, A. S., and A. G. Wollum, II. 1976. Comparative effects of methyl bromide, propylene oxide, and autoclave sterilization on specific soil characteristics. Turrialba. 26:351-355.

85. Martin, J. P., R. C. Baines, and A. L. Page. 1963. Observation on the occasional temporary growth inhibition of citrus seedlings following heat or fumigation treatment of soil. Soil Sci. 95:175-185.

86. Martin, J. P., D. G. Aldrich, W. S. Murphy, and G. R. Bradford. 1953. Effects of soil fumigation on growth and chemical composition of citrus plants. Soil Sci. 75:137-151.

87. Mason, E. 1928. Note on the presence of mycorrhiza in the roots of salt mash plants. New Phytol. 27:193-195.

88. Menge, J. A., C. K. Labanauskas, E. L. V. Johnson, and R. G. Pratt. 1978. Partial substitution of mycorrhizal fungi for phosphorus fertilization in the green house culture of citrus. Soil Sci. Soc. Amer. J. 42:926-930.

89. Menge, J. A., D. Steirle, D. J. Bagyaraj, E. L. V. Johnson, and R. T. Leonard. 1978. Phosphorus concentration in plants responsible for inhibition of mycorrhizal development. New Phytol. 80:575-578.

90. Moorman, T., and F. Brent Reeves. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid West: II. A bioassay to determine the effect of land disturbance on mycorrhizal population. Amer. J. Bot. 66:14-18.

91. Mosse, B. 1953. Fruitifications associated with mycorrhizal strawberry roots. Nature (London). 171: 974.

92. Mosse, B. 1956. Fructification of an Endogone species causing endotrophic mycorrhiza in fruit plants. Ann. Bot. (London) 20:349-362.

93. Mosse, B. 1957. Growth and chemical composition of mycorrhizal and non-mycorrhizal apples. Nature (London) 179:922-924.

94. Mosse, B. 1962. The establishment of vesicular-arbuscular mycorrhiza under aseptic conditions. J. Gen. MicroBiol. 27:509-520.

95. Mosse, B. 1963. Vesicular-arbuscular mycorrhiza: An extreme form of fungal adaptation. p. 146-170. In P. S. Nutman, and B. Mosse (eds) Symbiotic Associations. Cambridge University Press.

96. Mosse, B. 1972. Effects of different Endogone strains on growth of Paspalum notatum. Nature (London) 239: 221-223.

97. Mosse, B. 1973a. Advances in the study of vesicular-arbuscular mycorrhiza. Ann. Rev. Phytopath. 11:171-196.

98. Mosse, B. 1973b. Plant growth response to vesicular-arbuscular mycorrhiza: IV. In soils given additional phosphate. New Phytol. 72:127-136.

99. Mosse, B. 1975. Specificity in VA mycorrhiza. p. 469-489. In F. E. Sanders, B. Mosse, and P. B. Tinker (eds) Endomycorrhizas. Academic Press, London.

100. Mosse, B. 1977a. Plant growth response to vesicular-arbuscular mycorrhiza: X. Response of stylosanthes and maize to inoculation in unsterile soils. New Phytol. 78:277-288.

101. Mosse, B. 1977b. The role of mycorrhiza in legume nutrition in marginal soils. p. 275-295. In J. M. Vincent, A. S. Whitney, and J. Bosse (eds) Exploiting the Legume-Rhizobium Symbiosis in Tropical Agriculture. University of Hawaii Misc. Publ. no. 145.

102. Mosse, B. 1981. Vesicular-arbuscular mycorrhiza Research for Tropical Agriculture. Hawaii Institute of Tropical Agriculture and Human Resources, University of Hawaii Research Bulletin no. 194.

103. Mosse, B., and G. D. Bowen. 1968a. A key to the recognition of Endogone spore types. Trans. Brit. Mycol. Soc. 51:469-483.

104. Mosse, B., and G. D. Bowen. 1968b. The distribution of Endogone spores in some Australian and New Zealand soils and in an experimental field soil at Rothamsted. Trans. Brit. Mycol. Soc. 51:485-492.

105. Mosse, B., and D. S. Hayman. 1971. Plant growth response to vesicular-arbuscular mycorrhiza: II. In unsterilized field soils. New Phytol. 70:29-31.

106. Mosse, B., and D. S. Hayman. 1980. Mycorrhiza in Agricultural plants. p. 213-230. In P. Mikola (ed) Tropical Mycorrhiza Research. Oxford University Press, New York.

107. Mosse, B., D. S. Hayman, and J. Arnold. 1973. Plant growth response to vesicular-arbuscular mycorrhiza: V. Phosphate uptake by three plant species from P-deficient soils labelled with $^{32}P$. New Phytol. 72: 809-815.

108. Mosse, B., and G. W. Jones. 1968. Separation of Endogone spores from organic soil by differential sedimentation on gelatin column. Trans. Brit. Mycol. Soc. 51:604-608.

109. Mosse, B., C. Ll. Powell, and D. S. Hayman. 1976. Plant growth response to vesicular-arbuscular mycorrhiza. IX. Interaction between VA mycorrhiza, rock phosphate and symbiotic nitrogen fixation. New Phytol. 76:331-342.

110. Munns, D. N., and B. Mosse. 1980. Mineral nutrition of legume crops. p. 115-125. In R. J. Sumerfield, and A. H. Bunting (eds) Advances in legume Science. Royal Botanical Garden, Kew, England.

111. Murdoch, C. L., J. A. Jacobs, and J. W. Gerdemann. 1967. Utilization of phosphorus sources of different availability by mycorrhizal and non-mycorrhizal maize. Plant and Soil 27:329-334.

112. Nicolson, T. H. 1967. Vesicular-arbuscular--a universal plant symbiosis. Sci. Prog. Oxford. 55:561-581.

113. Nicolson, T. H. 1959. Mycorrhiza in the graminae: I. Vesicular-arbuscular endophytes, with special reference to the external phase. Trans. Brit. Mycol. Soc. 42:421-428.

114. Nicolson, T. H., and J. W. Gerdemann. 1968. Mycorrhizal Endogone species. Mycologia. 60:313-325.

115. Nicolson, T. H., and N. C. Schenck. 1979. Endogonaceous mycorrhizal endophytes in Florida. Mycologia. 71:178-198.

116. Ohms, R. E. 1957. A flotation method for collecting spores of Phycomycetous mycorrhizal parasite from soil. Phytopath. 47:751-752.

117. Owusu-Bennoah, E., and B. Mosse. 1979. Plant growth response to vesicular-arbuscular mycorrhiza: XI. Response of onions, lucerne and barley to field inoculation with two endophytes. New Phytol. 83:1-7.

118. Owusu-Bennoah, E., and A. Wild. 1979. Autoradiography of the depletion zone of phosphate around onion roots in the presence of vesicular-arbuscular mycorrhiza. New Phytol. 82:133-140.

119. Peyronel, B., B. Fassi, A. Fantana, and J. Trappe. 1969. Terminology of mycorrhizae. Mycologia. 61:410-411.

120. Phillips, J. M., and D. S. Hayman. 1979. Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 55:158-161.

121. Porter, W. M. 1979. The 'most probable number' method for enumerating infective propagules of vesicular-arbuscular mycorrhizal fungi in soil. Aust. J. Soil Res. 17:515-519.

122. Powell, C. Ll. 1976. Development of mycorrhizal infections from Endogone spore, and infected root segments. Trans. Brit. Mycol. Soc. 66:439-445.

123. Powell, C. Ll. 1977. Effect of several mycorrhizal fungi on clover growth in sterilized soils. N. Z. J. Agric. Res. 20:59-62.

124. Powell, C. Ll. 1980. Mycorrhizal infecitivity of eroded soils. Soil Biol. Biochem. 12:247-250.

125. Powell, C. Ll., and J. Sithamparanathan. 1977. Infection rates in grass and legume species by indigenous mycorrhizal fungi under field conditions. N. Z. J. Agric. Res. 20:489-494.

126. Ramirez, B. N., D. T. Mitchell, and N. C. Schenck. 1975. Establishment and growth effects of three vesicular-arbuscular mycorrhizal fungi on papaya. Mycologia 67:1039-1043.

127. Read, D. J., H. K. Koucheki, and J. Hodgson. 1976. Vesicular-arbuscular mycorrhiza in natural vegetation systems: I. The occurrence of infection. New Phytol. 77:641-653.

128. Redhead, J. F. 1977. Endotrophic mycorrhizas in Nigeria. Species of the Endogonaceae and their distribution. Trans. Brit. Mycol. Soc. 69:275-280.

129. Reeves, F. B., D. Wagner, T. Moorman, and J. Kiel. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid West: I. A comparison of incidence of mycorrhizae in severely disturbed vs natural ecosystems. Amer. J. Bot. 66:6-13.

130. Rhodes, L. H., and J. W. Gerdemann. 1975. Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions. New Phytol. 75:555-561.

131. Rhodes, L. H. 1979. P uptake by vesicular-arbuscular mycorrhizae. In Abstracts from the forth North American Conference on Mycorrhiza. Colorado State University, Fort Collins, Colorado.

132. Robinson, P. J., and R. K. Jones. 1972. The effect of phosphorus and sulphur fertilization on the growth and dry matter, nitrogen, phosphorus and sulphur in Townsville stylo (stylosanthes humilis) Aust. J. Agric. Res. 23:633-640.

133. Ross, J. P. 1971. Effect of phosphate fertilization on yield of mycorrhizal and non-mycorrhizal soybeans. Phytopath. 61:1400-1403.

134. Ross, J. P., and J. W. Gilliam. 1973. Effect of Endogone mycorrhiza on phosphorus uptake by soybeans from inorganic sources. Soil Sci. Soc. Amer. Proc. 37:237-239.

135. Ross, J. P., and J. A. Harper. 1970. Effect of Endogone mycorrhiza on soybean yield. Phytopath. 60:1552-1556.

136. Rovira, R. D. 1976. Studies in soil fumigation: I. Effects on ammonium, nitrate and phosphate in soil and on growth, nutrition and yield of wheat. Soil Biol. Biochem. 8:241-247.

137. Sabet, Y. 1939. Cotton mycorrhiza. Nature (London) 144:37.

138. Safir, G. R., J. S. Boyer, and J. W. Gerdemann. 1971. Mycorrhizal enhancement of water transport in soybeans. Science, Wash., 172:581-583.

139. Safir, G. R., J. S. Boyer, and J. W. Gerdemann. 1972. Nutrient status and mycorrhizal enhancement of water transport in soybeans. Plant Physiol. 49:700-703.

140. Sanders, F. E. 1975. The effect of foliar-applied phosphate on mycorrhizal infection of onion roots. p. 261-276. In F. E. Sanders, B. Mosse, and P. B. Tinker (eds) Endomycorrhizas. Academic Press, London.

141. Sanders, F. E., and P. B. Tinker. 1971. Mechanism of absorption of phosphate from soil by Endogone mycorrhizas. Nature (London) 233:278-279.

142. Sanders, F. E., and P. B. Tinker. 1973. Phosphate flow into mycorrhizal roots. Pestic. Sci. 4:385-395.

143. Sanni, S. O. 1976a. Vesicular-arbuscular mycorrhiza in some Nigeria soils and their effect on the growth of cowpea (Vigna unguiculata), tomato (Lycopersicum esculentum) and maize (Zea mays). New Phytol. 77: 667-671.

144. Sanni, S. O. 1976b. Vesicular-arbuscular mycorrhiza in some Nigerian soils. The effect of Gigaspora gigantea on the growth of rice. New Phytol. 77:673-674.

145. Schenck, N., and K. Kinson. 1971. Endotrophic vesicular-arbuscular mycorrhiza on soybean in Florida. Mycologia. 63:672-674.

146. Slankis, V. 1965. Termination of the symbiotic relationship in ectotrophic mycorrhizae as affected by high nitrogen concentration. Plant Physiol. suppl. 40:76.

147. Sondergaard, M., and S. Laegaard. 1977. Vesicular-arbuscular mycorrhiza in some aquatic vascular plants. Nature (London) 268:232-233.

148. Singh, B. R., and Y. Kanehiro. 1970. Effects of gamma irradiation on the available nitrogen status of soils. J. Sci. Fd. Agric. 21:61-64.

149. Sparling, G. P., and P. B. Tinker. 1975. Mycorrhiza in Pennine grassland. p. 545-560. In F. E. Sanders, B. Mosse, and P. B. Tinker (eds) Endomycorrhizas. Academic Press, London.

150. Stout, P. R., and R. Overstreet. 1950. Soil chemistry in relation to inorganic nutrition of plants. Ann. Rev. Plant Physiol. 1:305-342.

151. Strzemska, J. 1974. The occurrence and intensity of mycorrhizas in cultivated plants. Proc. 10th Intl. Cong. Soil Sci. Moscow 3:81-86.

152. Sutton, J. C., and G. L. Barron. 1972. Population dynamics of Endogone spores in soil. Can. J. Bot. 50:1907-1914.

153. Thaxter, R. 1922. A revision of the Endogonaceae. Proc. Amer. Acad. Arts Sci. 57:291-351.

154. Tinker, P. B. H. 1975. Effects of vesicular-arbuscular mycorrhizas on higher plants. Symp. Soc. Exp. Biol. 29:325-349.

155. Tsao, P. H. 1960. A serial dilution end point method for estimating disease potential of citrus Phytophthora in soil. Phytopath. 50:717-724.

156. Waidyanatha, U. P. De S., N. Yogaratnam, and W. A. Ariyaratne. 1979. Effects of mycorrhizal infection on growth and nitrogen fixation of Pueraria and Stylosanthes and uptake of phosphorus from two rock phosphates. New Phytol. 82:147-152.

157. Wilhelm, S. 1966. Chemical treatments and inoculum potential of soil. Ann. Rev. Phytopath. 4:53-78.

158. Wilkins, W. H., and G. C. M. Harris. 1944. Investigation into the production of bacteriostatic substances by fungi: XI. Examination of the larger Basiomycetes. Ann. Appl. Biol. 31:261-270.

159. William, T. D., and G. A. Salt. 1970. The effect of salt sterilants on the cereal cystnematode (Heterodera avenae Woll.), take all (Ophiobolus graminis Sacc.) and yields of spring wheat and barley. Ann. Appl. Biol. 66:329-338.

160. Yost, R. S., and R. L. Fox. 1979. Contribution of mycorrhizae to the P nutrition of crops on an oxisol. Agron. J. 71:903-908.

161. Yost, R. S., and R. L. Fox. 1982. Influence of mycorrhizae on the mineral contents of cowpea and soybean grown in an oxisol. Agron. J. 74:475-480.

162. Zag, B. 1964. The role of mycorrhizae in root diseases. Ann. Rev. Phytopath. 2:377-392.